TOMATO RIPENING IN LOW OXYGEN STORAGE WITH EMPHASIS ON ETHYLENE AND CARBON DIOXIDE PRODUCTION, AND CAROTENOID BIOSYNTHESIS

By

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DEDICATION

To my wife, Il-Young.

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Chairman: Dr. C. B. Hall
Major Department: Vegetable Crops

The ripening pattern of tomatoes (cv. Walter) in low oxygen (5% O_2 and 2.5% O_2 in N_2) resembled that of nonclimacteric fruits, *i.e.* rates of CO_2 and ethylene evolution remained very low without climacteric rise, and the color, after 9 to 12 days' delay, developed slowly to yield pink-red fruit. Tomatoes resumed the normal climacteric rise of respiration and ethylene production when transferred at weekly intervals to an air stream, and the color developed fully into deep orange-red.

Comparison of the cumulative values of CO_2 and ethylene production to reach certain color grades strongly implies a quantitative requirement for ethylene and accompanying respiration for tomato ripening.

The modification of fruit color from red to pink-red in low oxygen was attributed to changes in the composition of carotenes, *i.e.* more accumulation of β-carotene and lycopene occurred with diminished proportions of phytofluene, ζ-carotene, and proneurosporene. The yellowish orange-red color developing upon exposure to air was attributed to the synthesis of the skin flavonol and carotenes with increased proportions of phytofluene, ζ-carotene, and proneurosporene.

INTRODUCTION

This study was initiated to examine the ripening behavior of tomatoes in low oxygen storage. Preliminary tests revealed that in a low oxygen atmosphere tomato ripening was modified in ethylene and CO_2 production rate, in speed of coloring and softening, and in the quality of the fruit color.

Parsons *et al.* (1970) pointed out in their controlled atmosphere storage of mature-green tomatoes that tomatoes stored in 6% O_2 and 3% O_2 developed a dark pink and a light pink color at full ripeness, respectively; whereas fruits stored in air ripened to a full red color. Salunkhe and Wu (1973) reported that formation of lycopene and β-carotene of tomato fruits was inhibited by low oxygen storage, however, the final color of the fruits was not affected by low oxygen storage.

The primary objectives of the present investigation were to examine the relationships of the respiratory climacteric and ethylene production to tomato ripening by employing a low oxygen atmosphere system, and to study the modification of pigmentation of tomatoes in a low oxygen atmosphere through carotenoid analysis.

REVIEW OF THE LITERATURE

Effect of Oxygen, Ethylene, and Respiration on Fruit Ripening

The historical development of postharvest physiology of fruit ripening can be traced through a series of reviews of Biale (1960), Burg (1962), Hansen (1966), Pratt and Goeschl (1969), Abeles (1972), and Sacher (1973).

Fruit Ripening in Low Oxygen Storage

The effects of low oxygen in the storage atmosphere on fruit ripening has been extensively studied along with the development of controlled atmosphere storage (Kidd and West, 1945; Biale and Young, 1947; Young and Biale, 1962) and hypobaric storage (Burg and Burg, 1966).

When fruit, of several types, is stored in low oxygen concentration, the respiratory climacteric is considerably delayed and reduced in magnitude (Kidd and West, 1945; Biale, 1960), the production of ethylene is likewise restricted (Burg and Burg, 1965, 1969). However, when the bananas were treated with ethylene prior to or during low oxygen storage, they ripened normally without a respiratory climacteric. These results led Quazi and Freebairn (1970) to suggest that many of the changes and metabolic processes of ripening may not be completely determined by the climacteric respiratory rise, and that part of the rise may represent a wasted or unnecessary energy loss. They stressed

that ripening was always associated with exogenously supplied ethylene, irrespective of the absence or presence of the climacteric rise.

Hulme *et al.* (1971) studied apple ripening in air and low oxygen storage. In 3% O_2 storage, respiratory rate and ethylene production rate of either whole fruit or disks were low. When the fruit was transferred from 3% O_2 to air, there was a rapid adjustment to the normal (as observed in air) situation with increased respiration, ethylene production, protein synthesis and malate effect. They suggested that low oxygen either inhibits the continued development of the ethylene producing system and the events which ethylene sets in motion or a part of the system producing the "enzymes of ripening" is only slowly built up in low oxygen.

Fruit color of tomato is one of the most important criteria of ripeness and marketable quality. As the tomato fruit ripens, chlorophylls degrade, the yellow skin flavonol, quercitrin, is synthesized (Wu and Burrell, 1958), and flesh carotenoids are accumulated. Little work has been done on tomato skin flavonoids except some from a genetics standpoint (Fleming and Myers, 1937; LeRosen *et al.*, 1941) and the possible involvement of phytochrome (Piringer and Heinze, 1954).

Tomato carotenoids were extensively studied with genetic lines in an effort to elucidate their biosynthetic pathway (Porter and Lincoln, 1950; Tomes *et al.*, 1953). It generally has been accepted that the types of individual carotenes in a tomato fruit is genetically controlled, so that once the fruit starts ripening, carotenoid biosynthesis proceeds to give the characteristic fruit color.

However, Parsons *et al.* (1970) reported in their studies of the optimum conditions of controlled atmosphere storage that mature-green

tomatoes stored 3 weeks in 3% O_2, followed by 3 weeks in 6% O_2 were dark pink, and tomatoes stored the entire 6 weeks in 3% O_2 were only light pink; whereas fruits stored continuously in air, ripened to a full red color.

Salunkhe and Wu (1973) studied ripening and associated biochemical changes of tomatoes in low oxygen atmosphere (10% O_2, 3% O_2, and 1% O_2 at 12.8°C). They reported that formation of lycopene and β-carotene of tomato fruits was inhibited by low oxygen atmosphere storage. However, no significant differences were noted in lycopene and β-carotene content of control fruits after 33 days and low oxygen-treated fruits after 87 days. From this result they concluded that low oxygen atmosphere delayed ripening but did not affect the final color of the fruits.

The Climacteric: Significance in Fruit Ripening

Since Kidd and West (1925) demonstrated a climacteric rise in respiration in ripening fruit, and since Biale (1960) introduced the concept of climacteric and nonclimacteric fruits, much effort has been expended in seeking to define and explain the climacteric *per se* (Spencer, 1965). Biale (1969) concluded that the mechanism for energy generation is in full operation throughout the climacteric. Pratt and Goeschl (1969) also reached a similar conclusion that the respiratory pattern only reflects the integrated energy requirements of the various, more or less simultaneous but separate, processes of ripening. One weakness of this hypothesis, as Sacher (1973) points out, is the absence of evidence that the energy requirements for ripening of nonclimacteric fruits are less than those of climacteric fruits.

Considerable progress has been made in recent years in studying systems to find what components are involved in the various processes. Dostal and Leopold (1967) reported that ethylene stimulation of color development in tomatoes was prevented by gibberellic acid, but the stimulation of the respiratory climacteric was not. Frenkel *et al.* (1968) reported that cycloheximide inhibited ripening of pears but did not inhibit the respiratory climacteric. McGlasson *et al.* (1971) studied the effect of a number of inhibitors on banana ripening. Cycloheximide inhibited ripening but not the respiratory climacteric, indicating that the climacteric rise is not dependent upon and integrated with other aspects of ripening. On the other hand, compounds which inhibited the respiratory climacteric invariably inhibited ripening, indicating that some essential non-respiratory components of ripening are dependent on the respiration rise or on the factors which lead to it.

Hansen and coworkers (Hansen and Blanpied, 1968; Wang and Hansen, 1970; Wang and Mellenthin, 1972a) showed with pears of different stages of maturity that respiration, ethylene production, and softening were differentially affected by ethylene treatment. It was subsequently shown (Wang *et al.*, 1972b) that in pears at 85% maturity an internal ethylene concentration of 0.08 µl/l initiated softening, while 0.46 µl/l was required for initiation of the climacteric. Blanpied and Hansen (1968) also showed that pears ripened at low oxygen concentration (2.5% O_2) without an increase in respiration, Wang and Hansen (1970) suggested that the climacteric may have no unique function in ripening and that its biochemical significance and relationship to senescence is in need of reconsideration. Reid and Pratt (1972), after reviewing recent work and their own study on the effect of ethylene

on potato tuber respiration, concluded that the respiratory climacteric is not the primary event of ripening; instead it is a parallel event, induced by endogenous ethylene.

Ethylene and Fruit Ripening

The essentiality of ethylene for fruit ripening was demonstrated by Burg and Burg (1966) in a subatmospheric pressure study. Burg and Burg (1969) also studied the interaction of ethylene, oxygen and carbon dioxide and concluded that carbon dioxide antagonized most biological responses to ethylene by competitively inhibiting ethylene attachment to a metal-containing receptor, that ethylene production was inhibited at low oxygen concentrations, and that oxygen was required for ethylene action. Thus, low concentrations of oxygen retarded fruit ripening by inhibiting both ethylene production and action. Hulme *et al.* (1971) reached the same conclusion for apples in low oxygen storage.

In spite of the essentiality of ethylene for ripening, most researchers put emphasis on the role of ethylene as the trigger of ripening, rendering the role of the excess production of ethylene which accompanies ripening, the climacteric, and final senescence to be unknown (Pratt and Goeschl, 1969; McGlasson, 1970; Sacher, 1973). However, there is some indication that there exists a continuous need for ethylene in order that ripening may proceed after being initiated. Quazi and Freebairn (1970) showed that bananas, which had been preconditioned to ripen by a 24-hour exposure to ethylene, ripened earlier and more uniformly in low oxygen, than fruits not exposed to

ethylene. The delay of ripening in low oxygen tension could be counteracted partially by providing ethylene, as demonstrated with bananas (Hesselman and Freebairn, 1969), and apples (Hulme *et al.*, 1971).

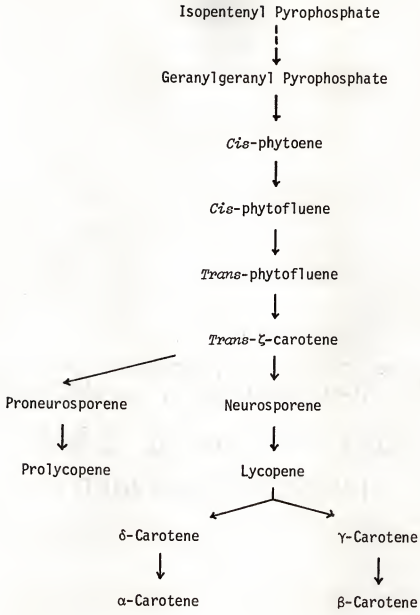
Carotenoid Biosynthesis

Biosynthetic Pathway of Carotenes

The first detailed pathway of carotene biosynthesis was proposed by Porter and Lincoln in 1950. It was primarily based on the analysis of carotenoid mutants of tomato and on the structure of the proposed intermediates. This biosynthetic pathway was modified by Porter and Anderson (1962, 1967), and later by Kushwaha *et al.* (1970) after a series of studies (Jungalwala and Porter, 1967; Kushwaha *et al.*, 1969; Subbarayan *et al.*, 1970) employing soluble enzyme systems obtained from plastids of spinach and tomato fruits for the conversion of labeled precursors to more unsaturated acyclic, monocyclic, and dicyclic carotenes.

The enzymic conversion of phytoene into lycopene involves the stereospecific loss of the 5-pro-R-hydrogen atom of mevalonate (Williams *et al.*, 1967; Walton *et al.*, 1969) and the 2-pro-S-hydrogen atom of mevalonate (Goodwin, 1971a) at each of 4 dehydrogenation steps. An isomerization occurs from *cis*-phytofluene to *trans*-phytofluene in the proposed pathway (Jungalwala and Porter, 1967; Qureshi *et al.*, 1974a).

The following is the carotenoid biosynthetic pathway proposed by Kushwaha *et al.* (1970).



In addition to the above pathway, *cis*-ζ-carotene was reported to exist between *trans*-ζ-carotene and proneurosporene (Qureshi *et al.*, 1974a, b), and α- and β-zeacarotene were proposed to be produced after cyclization of neurosporene (Goodwin, 1971a, b). The enzymic conversion of tritiated lycopene to cyclic carotenes by soluble preparations of spinach and tomato plastids was reported by Kushwaha *et al.* (1969). This con-

version of lycopene to cyclic carotenes was greater in an atmosphere of nitrogen than in air.

Trans-ζ-carotene has been recognized as a key intermediate in the biosynthesis of other carotenes since it is the branch point in the formation of poly-*cis* and *trans*-carotenes (Qureshi *et al.*, 1974a, b). *Trans*-ζ-carotene is present in large quantity with poly-*cis*-carotenes (proneurosporene and prolycopene) in tangerine genotype (tt) of tomato (Porter and Lincoln, 1950). In this genotype, ζ-carotene, proneurosporene and prolycopene are present. Introduction of gene T (in the presence of gene R) results in a conversion of these pigments of tangerine system into a lycopene - β-carotene system (Mackinney and Jenkins, 1952; Tomes *et al.*, 1953; Porter and Anderson, 1962). Zechmeister and Went (1948) were the first to interpret this in terms of a stereochemical effect. They suggested that whereas the biosynthesis of either all-*trans*-lycopene or prolycopene requires the dominant gene, R, the gene pair T and t determines the spatial configuration of the lycopene molecule. Porter and Anderson (1962) favored this idea that gene T controls the formation of an enzyme which converts *cis* isomers to *trans* isomers. One weakness of this interpretation is that the synthesis of all-*trans*-ζ-carotene in the absence of gene T cannot be explained by *cis*-*trans* isomerization.

Mackinney and Jenkins (1952) gave a different interpretation by suggesting that a possible role of gene T is in the dehydrogenase system. When carotenoid synthesis is great, as in RRtt, there is a partial failure of the dehydrogenase, and ζ-carotene and consequently proneurosporene accumulate. Porter and Anderson (1962) extended their

possible interpretation to suggest that this enzyme (which is controlled by gene T) must also affect the equilibrium of the reactions involved in the conversion of phytoene to lycopene, for the latter comprised over 80% of the carotenes found in red fruit. Porter and Anderson (1962) also suggested the possibility that gene T controls the formation of an enzyme which converts ζ-carotene to neurosporene and lycopene without the intermediate formation of proneurosporene and prolycopene.

Qualitative Modification of Carotenoid Composition

Qualitative composition of the carotenoids was reported to be changed by treatments with certain chemicals (Jensen *et al.*, 1958; Yokoyama *et al.*, 1972; Hsu *et al.*, 1974), anaerobic incubation and light (Lang and Rau, 1972), and callus cultures (Ulrich and Mackinney, 1970).

When a photosynthetic bacterium *Rhodospirillum rubrum* was incubated with diphenylamine (DPA), the net synthesis of the normal carotenoids (lycopene, P481, and spirilloxanthin) was completely arrested and a series of more saturated carotenenoids (phytoene, phytofluene, ζ-carotene and neurosporene) accumulated in the cells (Jensen *et al.*, 1958). When the DPA-grown cells were washed, resuspended in buffer and incubated anaerobically in the light for 24 hours, the quantity of the phytofluene, ζ-carotene and neurosporene steadily decreased, with a concomitant increase in the normally occurring pigments.

When citrus fruits which do not normally accumulate lycopene were treated with 2(4-chlorophenylthio)triethylamine hydrochloride

(CPTA), large amounts of lycopene accumulated (Yokoyama *et al.*, 1971, 1972). Hsu *et al.* (1974) reported 6 more amines which cause large increases in lycopene accumulation when placed on *Blakeslea trispora*. The modes of action of these amines appeared to be similar to that of CPTA.

When the mycelia of the fungus *Fusarium aquaeductum* were anaerobically incubated for 12 hours in the dark after 10 minutes in oxygen and darkness, the total carotenoid content was not changed, and all components (ζ-carotene, neurosporene, γ-carotene, lycopene, torulene, and neurosporaxanthin) remained unchanged except that γ-carotene increased at the expense of lycopene (Lang and Rau, 1972). When the 12-hour anaerobic incubation in the dark was preceeded by a 10-minute illumination in oxygen, the percentage of ζ-carotene increased at the expense of neurosporene and lycopene with other components remaining unchanged.

Ulrich and Mackinney (1970) studied carotenoid synthesis in the masses of callus derived from hypocotyls of the "Red Ghost" and "Tangerine" Tomatoes to test the hypothesis that the callus tissue should be able to produce the carotenoids typical of the genotype, since undifferentiated plant cells are presumably totipotent. The "Red Ghost" callus contained predominantly phytoene and phytofluene; the "Tangerine" callus had primarily ζ-carotene and prolycopene. Thus under their cultural conditions, carotenoids typical of the fruit rather than the leaf were produced, albeit in much reduced quantities.

In studies of carotene synthesis from [1-^{14}C]isopentenyl pryophosphate, [^{14}C]phytoene, and [^{14}C]lycopene by soluble enzyme

systems obtained from fruits of nine genetic selections, Papastephanou *et al.* (1973) found unexpected enzyme activities. Their analyses showed the presence of enzymes for the synthesis of phytoene in fruit of selections which do not synthesize significant amounts of this compound in naturally grown fruits. They have also shown the presence of enzymes for the synthesis of poly-*cis* compounds in two selections in which this was not expected, and the presence of enzymes for the cyclization of lycopene in fruits that form only small amounts of cyclic carotenes. They left unanswered the reasons for the discrepancies in the carotene composition that are observed between the fruits of field-grown tomato selections and cell-free enzyme preparations obtained from these fruits. However, they speculated that either inhibitors are present, cofactors are missing, or there are permeability barriers to substrate or cofactors.

Ultrastructure of Chromoplasts of Tomato Mutants

Harris and Spurr (1969a, b) followed the ultrastructural development of chromoplasts of three pigment lines of tomato mutants, low-pigment, high-beta, and red. In general, as the chromoplast develops from a chloroplast, the grana become disorganized and the thylakoids appear to separate at the partitions, and globules increase in size and number. They reported that β-carotene was presumably present largely in the globules. It crystallized into elongated or druce type forms which may distort the globules. On the other hand, lycopene aggregated on thylakoid membranes. Thylakoid plexes with a large regular lattice sometimes occurred in the chromoplasts of the high-beta mutant and

red tomato after the orange-red (firm-ripe) stage. Rosso (1967) reported the presence of the thylakoid plexes in chromoplasts of tangerine tomato cultivar.

Flavonoids of the Tomato Skin

The distinction between tomato flesh carotenoids and skin flavonoids was made quite early. Euler *et al.* (1931) noted in the dried skin of "Golden Queen" tomato a yellow flavonoid dye which was soluble in alkali. LeRosen *et al.* (1941) showed that two pairs of genes, R and r, causing red or yellow flesh color in tomatoes, and Y and y, producing yellow or colorless skin, are completely unrelated not only genetically but also biochemically.

Wu and Burrell (1958) were the first to critically identify the flavonoids of tomato skin. They reported that the fruit skins of three varieties of tomatoes, "Ponderosa," "Rutgers," and "Sunny Ray," contained naringenin and quercitrin. No flavonoids were detected in the peeled fruit. The leaf and petiole of these varieties contained quercitrin and rutin. Rivas and Luh (1968) detected naringenin and rutin in tomato paste. Quercitrin was not positively identified in these pastes. The differences in variety, sampling, extraction and isolation methods might have caused the contradicting results.

Little attention has been paid to the biochemistry and physiology of the tomato flavonoids in contrast to the extensive study of tomato carotenoids. The work of Piringer and Heinze (1954) has been one of the significant contributions to the tomato flavonoid study. Although they did not positively identify the flavonoids of the tomato cuticle

layer, they clearly demonstrated that synthesis of the yellow skin pigment was controlled by red:far-red light system. This pigment was produced in light-ripened fruits, but was absent in dark-ripened fruits. The threshold value for the light requirement to produce the yellow skin pigment was equivalent to that supplied by an incandescent-filament lamp somewhere between 0.0005 and 0.005 fc for one hour, or 0.03 or 0.3 fc for one minute per day during the ripening period. The induction of the flavonoid synthesis by red irradiation was reversed by the far-red irradiation but this reversal was not complete because far-red given after similar red treatments resulted in markedly less pigment formation but more than in the dark control.

MATERIALS AND METHODS

Experimental Design

Two experiments were devised to study the effect of storing mature-green tomatoes in low oxygen on their color grade, firmness, synthesis of carotenoids and flavonoids, and production of carbon dioxide and ethylene.

Experiment I. Effect of low oxygen concentration on color grade, and on CO_2 and ethylene production. Each of the gas mixtures used, namely, air, 5% O_2 in N_2 and 2.5% O_2 in N_2 streams (100 ml/min) was in a flow-through system of 8 one-gallon jars each containing 6 fruits. The gas streams were monitored by an infrared gas analyzer. Two gas streams were monitored by an infrared gas analyzer. Two jars from each low oxygen stream were transferred to an air stream at weekly intervals. Color grade and ethylene production were recorded every day, and CO_2 production was automatically recorded every 4 hours.

Experiment II. Effect of low oxygen concentration on color grade, firmness, and on carotenoid and flavonoid synthesis. Mature-green tomatoes were placed in 5-gallon jars through which flowed air, 5% O_2 in N_2 or 2.5% O_2 in N_2 streams (200 ml/min) to determine the effect on the coloration, softening, and on carotenoid and flavonoid synthesis of tomatoes. Duplicate samples of 3 tomatoes were taken at weekly intervals until the 7th week to see the effect in con-

tinuous low oxygen storage. To see the transfer effect, duplicate samples of 3 tomatoes for each sampling were transferred in air or hypobaric condition (127 mm-Hg) after being held in 5% O_2 and 2.5% O_2 for 7, 14, and 21 days. The transferred fruits were allowed to ripen in air or under hypobaric condition for 1, 2, 4, and 7 days. After color grading, firmness measurement and photographing on each sampling date, samples were put into freezer (-10°C) for further analysis.

Harvest and Handling

The tomato cultivar Walter (Strobel *et al.*, 1969) was used. Tomatoes were grown at the University of Florida Horticultural Unit at Gainesville. Harvest was made June 27, 1973. Mature-green fruits were selected for weight and size uniformity and absence of apparent defects. Special care was taken to prevent mechanical injury throughout harvesting, transporting, and washing. Fruits were placed in the different oxygen concentrations within 15 hours after harvest.

Low Oxygen Concentration System

Gas mixtures were made by means of a combination of capillary type flow meters. Nitrogen was supplied from 220 cubic foot high pressure cylinders and the pressure was reduced to 3 to 5 psi by means of a two-stage pressure reduction regulator. Air from the laboratory supply line was used as an air control and an oxygen source for the gas mixtures. This air supply contained about 0.03% CO_2,

however, the CO_2 level was negligible after dilution, and was considered to be satisfactory for the experimental purpose. Ethylene was not detected in any of the gas mixtures.

Figure 1 presents the flow chart for the low oxygen concentration and air treatment of tomato fruits. The system, except the infrared gas analyzer, was installed in a ripening room with a constant temperature (20°C). For "Experiment I" in which ethylene and CO_2 production were to be determined, the gas mixture was conducted through 1-gallon jars (F) containing 6 fruits and then to the infrared gas analyzer (I), for CO_2 determination, by means of 3-way solenoid valves (H). Gas samples for ethylene determination were taken with a 1-ml gas tight plastic syringe through the rubber tubing (L) at the outlet from the jar.

The flow rate of the gas streams (air, 5% O_2, and 2.5% O_2) flowing through each jar (F) was 100 ml/min measured at the outlet (K) of the infrared gas analyzer. The 5% O_2 and 2.5% O_2 concentrations (E) were obtained by first establishing the air (B) flow rates at the end point (K) at 23.8 ml/min (100 ml/min x 5/100 x 100/21) and 11.9 ml/min, respectively; and then by supplying N_2 (A) to make the final flow rate 100 ml/min. The calibration of the capillary pressure difference (D) of each air and N_2 supply line was controlled by the Swagelock fine needle valve (precision type) (C). The capillary pressures were calibrated beforehand for the cases when two jars each from 5% O_2 and 2.5% O_2 streams were transferred to an air stream at weekly intervals.

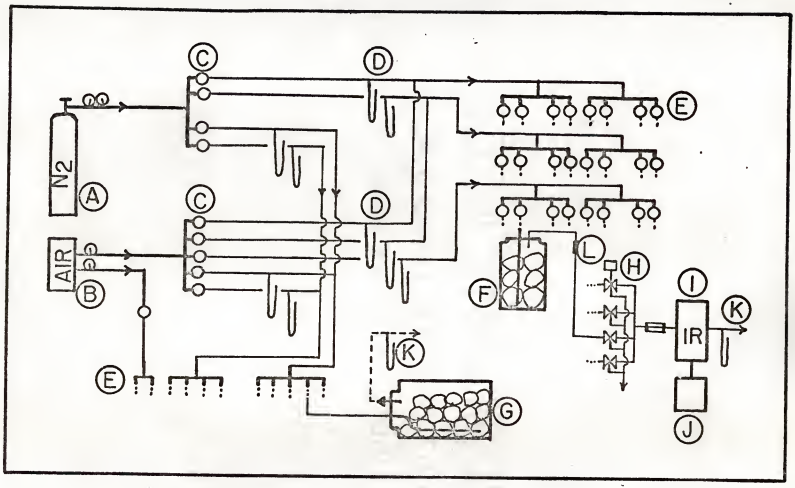


Figure 1. Schematic diagram of the apparatus for storage of tomatoes in air, 5% O_2, and 2.5% O_2 streams. (A) nitrogen; (B) air; (C) Swagelock fine needle valve (precision type); (D) capillary type gas flow meter; (E) stream lines of 5% O_2, 2.5% O_2, and air; (F) 1-gallon jar; (G) 5-gallon jar; (H) 3-way solenoid valve; (I) infrared gas analyzer; (J) recorder; (K) gas flow meter at the outlet; (L) rubber tubing for ethylene sampling.

For Experiment II in which color grade, firmness and pigment content were to be determined, the gas mixture (E) was conducted through 5-gallon jars (G) which contained about 50 fruits at the beginning. The mature-green fruits were prelabeled to designate the sequence of sampling dates and put into the 5-gallon jars with the sequential arrangement so that it would take only about 15 minutes to break the continuous low oxygen stream for weekly sampling. The flow rate of air, 5% O_2 and 2.5% O_2 streams was maintained at 200 ml/min (K).

Hypobaric Pressure System

The hypobaric pressure system was prepared by maintaining a pressure of 127 mm-Hg in four 10-liter vacuum desiccators with air flow through the apparatus at 250 ml/min (Burg and Burg, 1966). The system was installed in the ripening room at a constant temperature of 20°C. The air supply was water-saturated. Duplicate samples of tomatoes were removed from the 5-gallon jars of 5% O_2 and 2.5% O_2 after 7, 14, and 21 days of treatment, and then into the hypobaric storage system. Duplicates of 3 fruits from each treatment were removed from the hypobaric storage after 1, 2, 4, and 7 days. The color grade and firmness of the fruits were recorded.

Measurement and Determination

Color grade. A nondestructive color scoring method was employed instead of the objective method, such as Hunter color meter, because the fruits had to be scored in the jar, and the sample size

was limited to carotenoid analysis. A numerical value of color grade was assigned as follows for each fruit in a given sample at each observation period.

1 = Mature-green ; entirely green
2 = Breaker ; first appearance of coloring
3 = Turning ; transition phase with green and pink
4 = Pink ; entirely pink, no green
5 = Red ; fully red colored, including table ripe and soft ripe.

Firmness. Firmness was measured with a Cornell pressure tester (Hamson, 1952) using a 3/4-inch plunger and a 2000-gram weight for 5 seconds. The range of the instrument was 0 to 7, the higher the value the softer the fruit. Care was taken to align the fruit so that the plunger was applied over an inner wall as judged by external appearance.

Carbon dioxide. CO_2 was measured every 4 hours during the ripening studies with an automated gas analysis system employing a Beckman Model 215A infra-red CO_2 analyzer.

Ethylene. Ethylene was measured by using a HY-FY Aerograph, Model 600-D, gas chromatograph with a flame ionization detector. The column was 6' x 1/8" OD, copper tubing packed with activated alumina. The flow rate of hydrogen and carrier gas at the flame tip was 60 ml/min. Compressed air was supplied with a flow rate of 300 ml/min at the flame tip. Prepurified nitrogen was used as a carrier gas. The oven temperature was maintained at 100°C, and the injector temperature was 150°C. Ten ppm ethylene standard was used as reference gas for calibration at each time of measurement. A

rubber septum was present in the effluent line of fruit jar from which 0.5 ml gas samples were withdrawn into a 1.0 ml gas-tight plastic syringe. Ethylene production was determined once every morning. Sensitivity of ethylene detection was 5 ppb.

Carotenoid Analysis

Tomato Sample

On each sampling date labeled whole tomatoes were sealed in polyethylene bags and stored at -10°C until analyzed. For carotenoid analysis two fruits each from three different treatments were chosen. Half of each fruit was taken for the analysis. The corky part of the stem end, seeds and jelly-like parenchyma tissue were discarded before thawing. Skin tissue was carefully separated from the mesocarp when half thawed. Care was taken to remove any mesocarp tissue from the exocarp (skin tissue). After thawing, the fleshy mesocarp was macerated in a Waring blender for 3 minutes. Samples of from 20.0 to 40.0 g, according to the degree of color development were taken for carotenoid determination.

Pigment Extraction

The weighed homogenate was ground at high-speed in a Waring blender for 3 minutes with the addition of 40 ml of acetone-hexane (6:4, v/v) solvent, 0.5 - 1.0 g of $MgCO_3$ and approximately 5 grams of filter-aid (Celite 545). The homogenate was filtered on a Buchner funnel with suction. The residue on the filter paper was washed with a small volume of acetone and hexane until colorless.

Extraction was repeated until the pulp and the last filtrate were colorless. The filtrate was transferred to a 500 ml separatory funnel and washed several times with water to remove the acetone. This extract in hexane was saponified by occasional shaking with 1/8 volume methanol saturated with KOH. After 1 hour, the hypophase was discarded. The saponification was repeated briefly, and the hexane epiphase was washed twice with 100 ml of 90% methanol to remove the oxygenated carotenoids (Petracek and Zechmeister, 1956; Purchell, 1962; Meredith and Purcell, 1966). The hexane phase was washed free of alkali and methanol, and dried by filtering through an 18 x 100 mm column of anhydrous sodium sulfate. Then it was concentrated to about 3 ml using a rotary evaporator, quantitatively transferred to a 10 ml volumetric flask and stored refrigerated overnight.

Chromatography

Materials. The following materials were used.

Acetone and Hexane: Nanograde acetone and hexane of Mallinckrodt Chemical Co. were used without further purification.

Methanol: Analytical grade methanol was used.

Magnesia: Absorptive magnesium oxide, Sea Sorb 43 from Westvaco Chemical Divison was used.

Diatomaceous Earth: Hyflo Super Cel, Johns-Manville Corp.

Chromatographic Mixture: The media, Sea Sorb 43 and Hyflo Super Cel were previously activated at above 200°C for about 2 hours and cooled overnight in a desiccator before mixing in equal weight portions.

The mixing was accomplished by vigorously shaking the dry media in a 100 ml Ehrlenmeyer flask by hand for about 5 minutes.

Anhydrous Sodium Sulfate: Analytical reagent grade, granular, Scientific Product Co.

Column packing. The columns were packed with 60 ml each of dry media to make 18 x ca. 120 mm columns. A 5 mm layer of anhydrous sodium sulfate was added on top of the media and a cotton-wool plug was fitted in to a depth of about 1 cm to prevent surface disturbance. The columns were packed just before use.

Elution and pressure system. A pressure system with nitrogen pressure on top and vacuum suction on the bottom provided fast elution and sharp separation without causing any breakage or channeling of the column. The glass column had a 300 ml reservoir on top of the media and sintered glass on the bottom. Under this there was an outlet to an aspirator and a 20/24 ground glass joint end. Nitrogen pressure, regulated by a dual stage pressure regulator, was applied to the 6 columns by passing through "Tygon" tubing and rubber stoppers sealing the mouths of the columns. The rubber stoppers were easily removed after clamping off the nitrogen supply to facilitate occasional additions of elution solvent. The individual carotene fractions were collected at the bottom of the column in 250 ml ground neck Ehrlenmeyer flasks. The replacement of new flasks for the collection of subsequent fractions was done after releasing the vacuum and then gently disconnecting the ground glass joints of the flask from the column.

Pigment separation and elution. A 5 ml aliquot of the extract was evenly applied with a pipette on the surface of the absorptive

media. After the extract was quantitatively washed into the column, it was filled with 200 ml of hexane to elute phytoene and phytofluene. The column was developed by increasing the concentration of acetone in hexane stepwise up to 10% (Table 11). Phytoene, phytofluene, β-carotene, ζ-carotene, and proneurosporene were eluted in that order and collected as separate fractions. The γ-carotene, neurosporene and lycopene were eluted in that order with increasing additions of methanol, from 1% to 3%, in 10% acetone-hexane. The individual fractions were evaporated to dryness on a 40°C water bath with a rotary evaporator, and made up to a known volume (5 ml to 20 ml) in hexane.

Spectrophotometric Determination

Spectra of the carotenoids in hexane were obtained with a Bausch and Lomb Spectronic 200 UV scanning spectrophotometer. The individual pigment concentration was determined using known absorptivity according to Tomes (1963), and the equation of Davies (1965).

Tomato Skin Color Rating

Ratings of skin color were made using a scale of 1 to 5 with 1 colorless and 5 deep yellow orange.

RESULTS

Experiment I. Effect of Low Oxygen Concentration on Color Grade, and on CO_2 and Ethylene Production

Color Grade, CO_2 and Ethylene Production in Low Oxygen and After Transfer to Air

Color grade. Tomatoes colored normally in air reaching grade 5 11 days after harvest (Table 1, Fig. 2). Tomatoes in low oxygen atmosphere remained mature-green until they started to show incipient color 9 and 12 days after storage in 5% and 2.5% O_2, respectively. The time of the onset of the tomato coloring (Fig. 2) closely matched the time of ethylene production (Fig. 4). Color continued to develop very slowly until it reached the color grades 4.8 and 4.2 in 5% and 2.5% O_2, respectively, by the end of the experiment (35 days after harvest). The quality of the color and anatomical pattern of pigmentation were noticeably different from normal tomato ripening. The differences will be presented later on.

When tomatoes were transferred to air after being held in low oxygen for 1, 2, or 3 weeks, color developed rapidly and uniformly all over the fruit, and the time to full ripeness was less than in the controls as shown by the steeper slopes compared to the control (air) (Fig. 2). It took one day for coloring to start when tomatoes were transferred to air after a one week low oxygen storage. There was no lag period following 2 and 3 weeks of storage (Fig. 2).

Table 1. The color grade of "Walter" tomato fruit held at 20°C in 5% O_2, 2.5% O_2 and/or air.

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	2 wks	3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2 wks	3 wks
1	1.2	1.0	-	-	-	1.0	-	-	-
2	1.7	1.0	-	-	-	1.0	-	-	-
3	2.2	1.0	-	-	-	1.0	-	-	-
4	2.8	1.0	-	-	-	1.0	-	-	-
5	3.6	1.0	-	-	-	1.0	-	-	-
6	4.1	1.0	-	-	-	1.0	-	-	-
7	4.4	1.0	-	-	-	1.0	-	-	-
8	4.8	1.0	1.1	-	-	1.0	1.0	-	-
9	4.9	1.2	2.0	-	-	1.0	1.7	-	-
10	4.9	1.2	3.0	-	-	1.0	2.3	-	-
11	5.0	1.3	4.1	-	-	1.0	3.5	-	-
12	5.0	1.4	4.9	-	-	1.1	4.6	-	-
13	5.0	1.5	5.0	-	-	1.2	4.8	-	-
14	5.0	1.9	5.0	-	-	1.3	5.0	-	-
15	-	2.3	-	2.4	-	1.5	-	2.1	-
16	-	2.6	-	3.5	-	1.9	-	3.2	-
17	-	2.8	-	4.0	-	2.0	-	4.1	-
18	-	2.9	-	4.6	-	2.2	-	4.7	-
19	-	3.0	-	5.0	-	2.5	-	5.0	-
20	-	3.1	-	5.0	-	2.8	-	5.0	-
21	-	3.1	-	5.0	-	3.0	-	5.0	-
22	-	3.2	-	-	3.6	3.1	-	-	3.4
23	-	3.2	-	-	4.2	3.2	-	-	4.0
24	-	3.2	-	-	5.0	3.3	-	-	4.8
25	-	3.6	-	-	5.0	3.3	-	-	5.0
26	-	3.8	-	-	5.0	3.7	-	-	5.0
27	-	3.8	-	-	5.0	3.7	-	-	5.0
32	-	4.8	-	-	5.0	4.1	-	-	5.0
35	-	4.8	-	-	5.0	4.2	-	-	5.0

Note: The color grade goes from 1 to 5 with 1 being green and 5 being red.

*Harvest day = June 27, 1973.

Table 1. Continued.

(Standard Deviations)

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
1	0.4	0.0	-	-	-	0.0	-	-	-
2	0.9	0.0	-	-	-	0.0	-	-	-
3	1.2	0.0	-	-	-	0.0	-	-	-
4	1.4	0.0	-	-	-	0.0	-	-	-
5	1.2	0.0	-	-	-	0.0	-	-	-
6	1.0	0.0	-	-	-	0.0	-	-	-
7	0.9	0.0	-	-	-	0.0	-	-	-
8	0.5	0.0	0.3	-	-	0.0	0.0	-	-
9	0.4	0.4	0.6	-	-	0.0	0.7	-	-
10	0.3	0.4	0.7	-	-	0.0	1.1	-	-
11	0.1	0.6	0.5	-	-	0.2	0.9	-	-
12	0.0	0.8	0.3	-	-	0.3	0.5	-	-
13	0.0	0.8	0.0	-	-	0.5	0.4	-	-
14	0.0	0.8	0.0	-	-	0.6	0.0	-	-
15	-	0.8	-	0.9	-	0.8	-	0.9	-
16	-	0.9	-	0.7	-	0.9	-	0.6	-
17	-	0.7	-	0.4	-	0.9	-	0.5	-
18	-	0.7	-	0.5	-	0.9	-	0.5	-
19	-	0.6	-	0.0	-	0.8	-	0.0	-
20	-	0.7	-	0.0	-	0.6	-	0.0	-
21	-	0.6	-	0.0	-	0.5	-	0.0	-
22	-	0.6	-	-	0.8	0.3	-	-	0.7
23	-	0.6	-	-	0.4	0.4	-	-	0.4
24	-	0.6	-	-	0.0	0.5	-	-	0.4
25	-	0.7	-	-	0.0	0.5	-	-	0.0
26	-	0.6	-	-	0.0	0.8	-	-	0.0
27	-	0.6	-	-	0.0	0.8	-	-	0.0
32	-	0.4	-	-	0.0	0.8	-	-	0.0
35	-	0.4	-	-	0.0	0.7	-	-	0.0

*Harvest day = June 27, 1973.

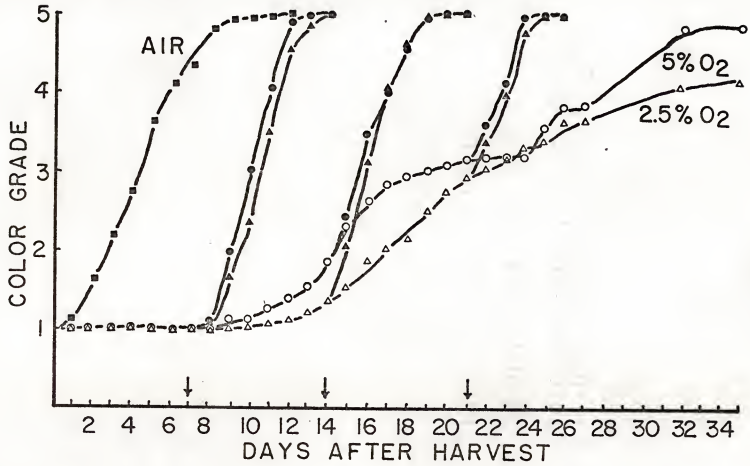


Figure 2. Color development of tomatoes in air, 5% O_2, and 2.5% O_2 at 20°C. Arrows indicate transfer of fruit from low oxygen to air. The color grade goes from 1 to 5 with 1 being green and 5 being red.

Carbon dioxide production. Tomatoes showed a typical respiratory climacteric when ripened in air (Fig. 3). In low oxygen atmosphere, the rate of CO_2 production decreased to a minimum in 3 days and remained low (Table 2, Fig. 3). The changes in CO_2 production rate (Fig. 3) were closely related to those of ethylene production rate (Fig. 4) in air and in low oxygen atmosphere. The rate of CO_2 production increased slightly from the 8th and 14th day in 5% and 2.5% O_2, respectively, corresponding to the onset of ethylene production.

When tomatoes were transferred to air after being held in low oxygen for 1, 2, or 3 weeks, the rates of CO_2 production (Table 2, Fig. 3) increased rapidly to reach a peak and then declined. The magnitudes of increase in CO_2 production rate upon transfer to air were different depending upon the oxygen level and duration of storage (Fig. 3). The peak rates of CO_2 production when tomatoes were transferred to air after one week in low oxygen were 11.88 ml/kg-hr for 5% O_2 and 12.16 ml/kg-hr for 2.5% O_2 which were comparable to the peak of 12.10 ml/kg-hr reached by the control (continuous air) (Table 2, Fig. 3). However, the peaks of CO_2 production rate obtained in air after 2 weeks in 5% and 2.5% O_2 were only 8.83 ml/kg-hr (73% of control) and 9.28 ml/kg-hr (77% of control), respectively. Carbon dioxide production showed a bimodal rate curve when tomatoes were transferred to air after a 3-week storage in low oxygen atmosphere. Tomatoes stored in 5% O_2 showed double peaks at 8.07 ml/kg-hr (67% of control) and 9.40 ml/kg-hr (77% of control). The response of CO_2 production rate to the transfer to air was rapid without the lag period observed in the corresponding cases of color grade and ethylene. The rate increased

Table 2. Carbon dioxide production (ml CO_2/kg-hr) by "Walter" tomato fruit held at 20°C in 5% O_2, 2.5% O_2 and/or air.

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
1	7.52	6.19	-	-	-	5.78	-	-	-
2	8.12	4.19	-	-	-	4.26	-	-	-
3	10.43	3.34	-	-	-	3.22	-	-	-
4	11.71	2.94	-	-	-	3.02	-	-	-
5	11.83	3.28	-	-	-	3.15	-	-	-
6	12.10	3.19	-	-	-	2.94	-	-	-
7	11.39	3.20	-	-	-	2.83	-	-	-
8	10.45	3.79	5.60	-	-	3.12	5.63	-	-
9	9.66	3.93	8.26	-	-	2.72	7.48	-	-
10	8.93	3.58	10.92	-	-	2.61	9.91	-	-
11	8.39	3.71	11.88	-	-	2.50	11.20	-	-
12	8.09	3.44	11.31	-	-	2.73	12.16	-	-
13	7.86	3.30	10.59	-	-	2.45	11.32	-	-
14	7.81	3.38	9.59	-	-	2.50	11.00	-	-
15	-	3.56	-	6.68	-	2.68	-	5.38	-
16	-	3.86	-	8.49	-	2.90	-	8.34	-
17	-	3.47	-	8.83	-	3.06	-	9.28	-
18	-	3.23	-	8.67	-	3.20	-	8.91	-
19	-	3.54	-	8.40	-	3.15	-	8.37	-
20	-	3.87	-	8.01	-	3.09	-	7.78	-
21	-	3.66	-	8.07	-	3.17	-	7.35	-
22	-	3.92	-	-	6.74	4.64	-	-	6.44
23	-	4.37	-	-	8.32	3.82	-	-	7.83
24	-	4.85	-	-	8.92	3.42	-	-	8.07
25	-	4.16	-	-	8.83	3.35	-	-	7.86
26	-	4.32	-	-	11.54	3.66	-	-	9.40
27	-	4.70	-	-	11.08	3.48	-	-	7.75
28	-	3.74	-	-	10.38	3.13	-	-	7.02

*Harvest day = June 27, 1973.

Table 2. Continued.

(Standard Deviations)

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
1	1.13	1.90	-	-	-	1.09	-	-	-
2	1.25	0.36	-	-	-	0.57	-	-	-
3	1.41	0.30	-	-	-	0.27	-	-	-
4	1.41	0.21	-	-	-	0.32	-	-	-
5	0.94	0.44	-	-	-	0.45	-	-	-
6	0.86	0.27	-	-	-	0.36	-	-	-
7	0.94	0.23	-	-	-	0.34	-	-	-
8	0.66	0.22	0.46	-	-	0.26	0.81	-	-
9	0.61	0.57	0.73	-	-	0.30	0.61	-	-
10	0.43	0.33	0.92	-	-	0.27	0.58	-	-
11	0.40	0.51	0.17	-	-	0.28	0.51	-	-
12	0.46	0.22	0.43	-	-	0.42	1.56	-	-
13	0.43	0.19	0.46	-	-	0.22	1.29	-	-
14	0.51	0.23	0.44	-	-	-	-	-	-
15	-	0.20	-	0.57	-	0.38	-	0.97	-
16	-	0.34	-	0.30	-	0.50	-	0.74	-
17	-	0.25	-	0.14	-	0.58	-	0.74	-
18	-	0.24	-	0.38	-	0.57	-	0.70	-
19	-	0.25	-	0.57	-	0.51	-	0.61	-
20	-	0.61	-	0.72	-	0.47	-	0.58	-
21	-	0.30	-	0.75	-	0.44	-	0.04	-
22	-	0.56	-	-	0.68	0.42	-	-	0.35
23	-	0.32	-	-	0.76	0.17	-	-	0.33
24	-	0.51	-	-	0.62	0.83	-	-	0.19
25	-	0.28	-	-	0.62	0.09	-	-	0.17
26	-	0.83	-	-	3.22	0.45	-	-	1.89
27	-	0.37	-	-	2.58	0.21	-	-	0.08
28	-	0.25	-	-	3.34	0.17	-	-	0.12

*Harvest day = June 27, 1973.

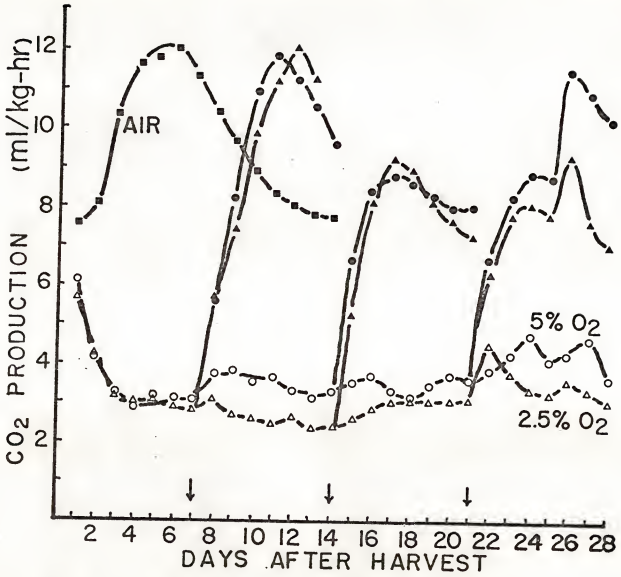


Figure 3. CO_2 production by tomatoes in air, 5% O_2, and 2.5% O_2 at 20°C. Arrows indicate transfer of fruit from low oxygen treatment to air.

from 75 to 98% in the first day following transfer from 5% O_2 and from 99 to 115% following 2.5% O_2.

Ethylene production. Fruits held in air produced ethylene at a rate of 0.48 µl/kg-hr on the first day. This rate was 8.2% of the highest rate of 5.83 µl/kg-hr produced on the eighth day in air (Table 3). The greatest increase was between 2 and 4 days with the increase of rates slowing between 4 and 8 days (Fig. 4). There was little difference in rate between 7, 8, and 9 days. The rate declined from the eighth day until the thirteenth day after which it increased abruptly on the fourteenth day to reach 6.16 µl/kg-hr. This increase presumably indicates the microbial infection of the fruit.

No ethylene was detected in fruits held in 5% O_2 until the eighth day when the production was 0.58 µl/kg-hr. The rate increased somewhat erratically and reached a rate of 1.84 µl/kg-hr on the twenty-third day, after which a slow decline set in. Thus, the highest rate for fruits held continuously at 5% O_2 was only 31.6% of the highest rate of fruits held in air.

Fruits held 1 week at 5% O_2 began to produce ethylene within 1 day after being transferred to air. The rate of ethylene production was 0.39 µl/kg-hr which was 4.9% of the highest rate of 7.93 µl/kg-hr produced on day 12 (5 days after transfer to air). The highest rate attained by this treatment, 7.93 µl/kg-hr was 36.0% more than the highest rate of fruits held in air (5.83 µl/kg-hr) and occurred 3 days earlier (Tables 3 and 4).

Fruits transferred to air after 2 weeks at 5% O_2 produced ethylene at the rate of 2.11 µl/kg-hr on the first day in air (day 15) which was

Table 3. Ethylene production (µl C_2H_4/kg-hr) by "Walter" tomato fruit held at 20°C in 5% O_2, 2.5% O_2 and/or air.

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
1	0.48	0.00	-	-	-	0.00	-	-	-
2	1.25	0.00	-	-	-	0.00	-	-	-
3	3.04	0.00	-	-	-	0.00	-	-	-
4	4.42	0.00	-	-	-	0.00	-	-	-
5	4.70	0.00	-	-	-	0.00	-	-	-
6	5.00	0.00	-	-	-	0.00	-	-	-
7	5.68	0.00	-	-	-	0.00	-	-	-
8	5.83	0.58	0.39	-	-	0.08	0.29	-	-
9	5.52	0.27	1.99	-	-	0.00	1.15	-	-
10	5.02	0.32	6.27	-	-	0.04	4.06	-	-
11	4.97	0.50	7.74	-	-	0.00	5.07	-	-
12	4.68	0.54	7.93	-	-	0.11	5.67	-	-
13	4.48	0.61	5.54	-	-	0.15	4.52	-	-
14	6.16	0.69	5.27	-	-	0.22	4.62	-	-
15	-	0.71	3.53	2.11	-	0.33	4.22	1.10	-
16	-	1.19	3.70	5.01	-	0.47	5.49	4.76	-
17	-	0.79	2.44	4.47	-	0.55	3.57	4.36	-
18	-	0.90	2.75	4.62	-	0.67	4.07	4.40	-
19	-	1.00	-	4.00	-	0.70	-	3.90	-
20	-	1.25	2.84	3.71	-	0.82	5.71	3.44	-
21	-	1.30	-	3.00	-	1.10	-	3.00	-
22	-	1.37	-	-	4.21	1.46	-	-	3.55
23	-	1.84	-	-	5.88	1.20	-	-	5.79
24	-	1.46	-	-	5.80	0.99	-	-	4.56
25	-	1.20	-	-	5.87	0.90	-	-	4.60
26	-	1.01	-	-	2.49	0.83	-	-	4.89
27	-	1.04	-	-	2.24	0.81	-	-	3.42
28	-	1.08	-	-	1.88	0.85	-	-	3.15

*Harvest day = June 27, 1973.

Table 3. Continued.

(Standard Deviations)

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
1	0.29	0.00	-	-	-	0.00	-	-	-
2	1.34	0.00	-	-	-	0.00	-	-	-
3	1.72	0.00	-	-	-	0.00	-	-	-
4	2.34	0.00	-	-	-	0.00	-	-	-
5	-	0.00	-	-	-	0.00	-	-	-
6	1.33	0.00	-	-	-	0.00	-	-	-
7	1.23	0.00	-	-	-	0.00	-	-	-
8	0.87	0.24	0.01	-	-	0.08	0.04	-	-
9	0.61	0.10	0.42	-	-	0.00	0.08	-	-
10	0.39	0.13	2.19	-	-	0.09	1.66	-	-
11	0.61	0.20	0.89	-	-	0.00	2.03	-	-
12	0.42	0.31	0.37	-	-	0.09	1.74	-	-
13	0.88	0.26	0.30	-	-	0.09	1.45	-	-
14	2.60	0.22	1.22	-	-	0.01	0.02	-	-
15	-	0.36	0.00	0.21	-	0.05	0.60	0.31	-
16	-	0.15	0.00	0.38	-	0.08	0.49	0.14	-
17	-	0.08	0.00	0.92	-	0.08	0.74	1.08	-
18	-	0.06	0.00	1.29	-	0.11	0.84	1.36	-
19	-	-	-	-	-	-	-	-	-
20	-	0.13	0.00	1.34	-	0.15	3.18	0.50	-
21	-	-	-	-	-	-	-	-	-
22	-	0.00	-	-	0.79	0.04	-	-	1.35
23	-	0.00	-	-	0.81	0.04	-	-	0.25
24	-	0.00	-	-	1.08	0.08	-	-	0.88
25	-	0.00	-	-	1.24	0.15	-	-	0.19
26	-	0.00	-	-	3.53	0.05	-	-	0.23
27	-	0.00	-	-	3.17	0.07	-	-	0.12
28	-	0.00	-	-	2.66	0.11	-	-	0.67

*Harvest day = June 27, 1973.

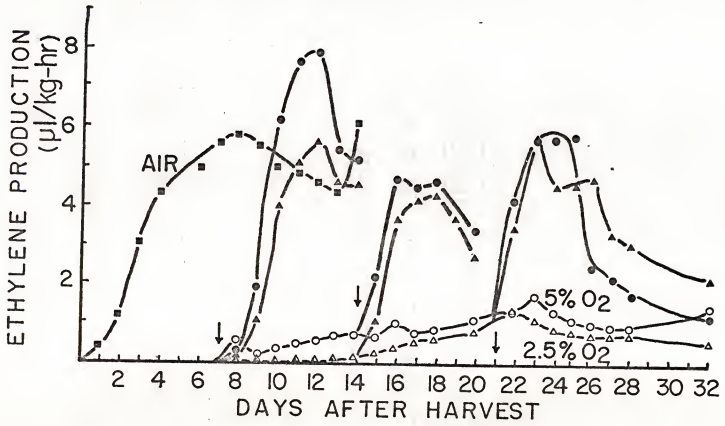


Figure 4. Ethylene production by tomatoes in air, 5% O_2, and 2.5% O_2 at 20°C. Arrows indicate transfer of fruit from low oxygen treatment to air.

Table 4. Maximum rate of ethylene production of tomatoes at 20°C in 5% O_2, 2.5% O_2 and/or air.

Treatment		Maximum rate	Days after transfer to air
		µl/kg-hr	
Control	(air)	5.83	8
5% O_2	1 wk	7.93	5
	2 wks	5.01	2
	3 wks	5.88	2
2.5% O_2	1 wk	5.67	5
	2 wks	4.76	2
	3 wks	5.79	2

206% greater than the rate of 0.69 µl/kg-hr on the day of transfer. The first-day rate (2.11 µl/kg-hr) was 42% of the highest rate. The highest rate obtained, 5.01 µl/kg-hr on the second day in air (day 16), was only 86% of the highest rate of fruits held only in air (Table 4).

A 224% increase in rate (from 1.30 to 4.21 µl/kg-hr) occurred in the first day after transfer to air following 3 weeks in 5% O_2. The highest rate, 5.88 µl/kg-hr, equalled the highest rate obtained in the controls and this occurred on the second day after transfer to air although there was no difference between the second, third and fourth days after transfer (days 23, 24, and 25). The first day rate was 72% of the highest rate.

Thus, the rate of ethylene production reached a peak in a shorter time after transfer to air from 5% O_2 as the duration in 5% O_2 increased. Also the rate of ethylene production increased markedly during the first day in air as the duration in 5% O_2 increased.

No ethylene was detected in fruits held in 2.5% O_2 until the eighth day when the production was 0.08 µl/kg-hr. However, ethylene was not detected again on day nine and eleven. The rate began increasing from day 12 (0.11 µl/kg-hr) and reached a rate of 1.46 µl/kg-hr on the twenty-second day followed by a slow decline. Thus, the highest rate for fruits held continuously at 2.5% O_2 was only 25.0% of the highest rate of fruits held in air (Table 3).

Fruits transferred to air after 1 week in 2.5% O_2 began to produce ethylene within 1 day at the rate of 0.29 µl/kg-hr. This rate was 5.1% of the highest rate of 5.67 µl/kg-hr produced on day 12 (5 days after transfer to air). The highest rate attained by this treatment, 5.67

µl/kg-hr was 2.7% less than the highest rate of fruits held in air (5.83 µl/kg-hr) and occurred 3 days earlier (Tables 3 and 4).

Fruits transferred to air after 2 weeks in 2.5% O_2 produced ethylene at the rate of 1.10 µl/kg-hr on the first day in air (day 15) which was 400% greater than the rate of 0.22 µl/kg-hr on the day of transfer. The first-day rate (1.10 µl/kg-hr) was 23% of the highest rate. The highest rate of 4.76 µl/kg-hr on the second day in air (day 16) was only 82% of the highest rate of fruits held only in air.

A 223% increase in rate (from 1.10 to 3.55 µl/kg-hr) occurred in the first day after transfer to air following 3 weeks in 2.5% O_2. The highest rate, 5.79 µl/kg-hr equalled the highest rate in air only and occurred on the second day after transfer to air. The first day rate was 61% of the highest rate.

Thus, the rate of ehtylene production reached a peak in a shorter time after transfer to air from 2.5% O_2 as the duration in 2.5% O_2 increased. Also the rate of ethylene production increased markedly during the first day in air as the duration in 2.5% O_2 increased.

Cumulative CO_2 and Ethylene Production

Cumulative CO_2 production. Tomatoes colored in low oxygen without a climacteric rise of respiration, suggesting that the respiratory climacteric may not be directly related to fruit coloring (Figs. 2 and 3). However, even though the rate of CO_2 production remained low in low oxygen atmosphere, the cumulative amount of CO_2 produced increased steadily throughout the period (Table 5, Fig. 5) and the overall pattern

Table 5. Cumulative values of hourly rates (ml/kg-hr) of carbon dioxide produced by "Walter" tomatoes at 20°C in 5% O_2, 2.5% O_2 and/or air for various periods.

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
				ml/kg fr. wt.					
1	7.5	6.2	-	-	-	5.8	-	-	-
2	15.6	10.4	-	-	-	10.0	-	-	-
3	26.1	13.7	-	-	-	13.3	-	-	-
4	37.8	16.7	-	-	-	16.3	-	-	-
5	49.6	19.9	-	-	-	19.4	-	-	-
6	61.7	23.1	-	-	-	22.4	-	-	-
7	73.1	26.3	-	-	-	25.2	-	-	-
8	83.6	30.1	31.9	-	-	28.3	30.8	-	-
9	93.2	34.1	40.2	-	-	31.0	38.3	-	-
10	102.1	37.6	51.1	-	-	33.7	48.2	-	-
11	110.5	41.3	63.0	-	-	36.2	59.4	-	-
12	118.6	44.8	74.3	-	-	38.9	71.6	-	-
13	126.5	48.1	84.9	-	-	41.3	82.9	-	-
14	134.3	51.5	94.5	-	-	43.8	93.9	-	-
15	-	55.0	-	58.1	-	46.5	-	49.2	-
16	-	55.9	-	66.6	-	49.5	-	57.6	-
17	-	62.4	-	75.5	-	52.5	-	66.8	-
18	-	65.6	-	84.1	-	55.7	-	75.7	-
19	-	69.1	-	92.5	-	58.8	-	84.1	-
20	-	73.0	-	100.5	-	61.9	-	91.9	-
21	-	76.7	-	108.6	-	65.1	-	99.2	-
22	-	80.6	-	-	83.4	69.7	-	-	71.5
23	-	84.9	-	-	91.7	73.5	-	-	79.4
24	-	89.8	-	-	100.6	77.0	-	-	87.4
25	-	94.0	-	-	109.5	80.3	-	-	95.3
26	-	98.3	-	-	121.0	84.0	-	-	104.7
27	-	103.0	-	-	132.1	87.5	-	-	112.4
28	-	106.7	-	-	142.5	90.6	-	-	119.5

Note: Cumulative values are derived from Table 2 by adding the hourly rates (ml/kg-hr) of CO_2 produced. Multiply by 24 to get cumulative daily production.

*Harvest day = June 27, 1973.

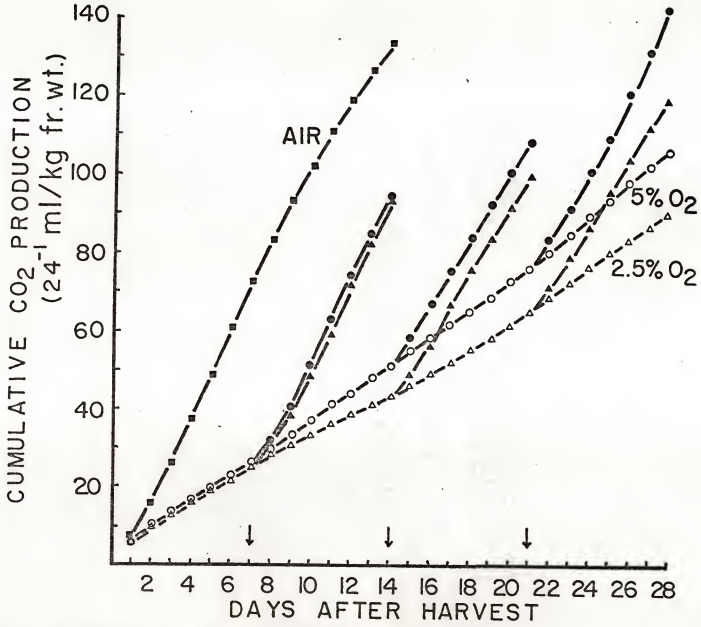


Figure 5. Cumulative values of hourly ratesof CO_2 produced by tomatoes at 20°C in 5% O_2, 2.5% O_2 and/or air. Multiply cumulative hourly rates by 24 to get cumulative daily production. Arrows indicate transfer of fruit from low oxygen treatment to air.

of the cumulative CO_2 production (Fig. 5) corresponded to that of color grade (Fig. 2).

The cumulative amounts of CO_2 produced during 7 days in air from the beginning or after transfer from low oxygen storage are given in Table 6. The cumulative amount of CO_2 produced in air (control) was 73.10 x 24 and 66.19 x 24 ml/kg for the first and second week, respectively. The corresponding amounts during 7 days in air following 1, 2, and 3 weeks in 5% O_2 were 68.15 x 24, 57.15 x 24, and 65.81 x 24 ml/kg, respectively. Following 1, 2, and 3 weeks in 2.5% O_2 the amounts were 68.70 x 24, 55.41 x 24, 54.37 x 24 ml/kg for 7 days in air (Table 6).

Although tomatoes had been exposed to different periods in low oxygen and in air after transfer before attaining color grade 5, the cumulative amount of CO_2 fell into a narrow range of values, *i.e.* 2.02 to 2.42 l/kg (Table 7). When tomatoes were ripened in air, 2.65 l/kg of CO_2 were produced by the time color grade 5 was reached. When tomatoes were held in 5% and 2.5% O_2 for 27 days they attained color grade 3.8 and 3.7, respectively, but the cumulative CO_2 production reached 2.47 and 2.10 l/kg, respectively which are similar to values obtained with color grade 5 in other treatments.

These cumulative data of CO_2 production indicate that even though a respiratory climacteric did not develop and the CO_2 production rate remained low in low oxygen atmosphere, enough CO_2 (respiration) was produced to support color development.

Cumulative ethylene production. Ethylene production of tomatoes in low oxygen atmosphere was delayed 8 to 12 days, depending upon

Table 6. Cumulative values of hourly rates of CO_2 and ethylene produced during 7 days in air after transfer from low oxygen storage.

Treatment		CO_2 in air		Ethylene in air	
		1st wk	2nd wk	1st wk	2nd wk
		ml/kg		µl/kg	
Control	(air)	73.10	66.19	24.57	36.66
5% O_2	1 wk	68.15		34.55	
	2 wks	57.15		26.92	
	3 wks	65.81		28.37	
2.5% O_2	1 wk	68.70		25.30	
	2 wks	55.41		24.96	
	3 wks	54.37		28.50	

Note: Cumulative values are derived from Tables 2 and 3 by adding hourly rates of CO_2 (ml/kg-hr) and ethylene (µl/kg-hr) produced. Multiply by 24 to get cumulative daily production.

Table 7. Cumulative amount of CO_2 and ethylene produced to reach a certain color grade by tomatoes held in air or low oxygen followed by air.

Oxygen level	Days in O_2	Days in air	Color grade	CO_2	Ethylene
				l/kg	µl/kg
Air	0	11	5.0	2.65	1101.84
	0	9	4.9	2.24	862.08
	0	8	4.8	2.01	729.60
5% O_2	7	6	5.0	2.04	716.64
	14	5	5.0	2.22	569.28
	21	3	5.0	2.42	636.96
2.5% O_2	7	7	5.0	2.27	609.12
	14	5	5.0	2.02	461.28
	21	4	5.0	2.29	569.76
5% O_2	27	0	3.8	2.47	445.68
2.5% O_2	27	0	3.7	2.10	274.32

the oxygen concentration. Ethylene production then began and slowly increased through day 22 or 23 after which the rate began to decrease (Fig. 4). However, the cumulative amount of ethylene produced increased steadily throughout the low oxygen period (Table 8, Fig. 6). The overall pattern of the cumulative production of ethylene (Fig. 6) corresponded with those of color grade (Fig. 2) and CO_2 (Fig. 5). The cumulative values of ethylene production during 7 days in air from the beginning or after transfer from low oxygen storage are given in Table 6. The cumulative amount of ethylene production in air (control) was 24.57 x 24 and 36.66 x 24 µl/kg for the first and second week, respectively. The corresponding amounts during 7 days in air following 1, 2, and 3 weeks in 5% O_2 were 34.55 x 24, 26.92 x 24, and 28.37 x 24 µl/kg, respectively. Following 1, 2, and 3 weeks in 2.5% O_2, the amounts were 25.30 x 24, 24.96 x 24 and 28.50 x 24 µl/kg for 7 days in air (Table 6).

The amount of ethylene accumulated to the time fruits reached color grade 5 ranged from 461.28 to 716.64 µl/kg for the low oxygen treatments transferred to air (Table 7). The mean value was 640.96 for 5% O_2 and 546.72 for 2.5% O_2. The 7 day at low oxygen value was highest at each oxygen level with the 14-day value lowest. By the time fruit held in air attained a color grade of 5, 1101.84 µl/kg of ethylene had been produced which is about 72% greater than the mean for 5% O_2 plus air, and 102% greater than the mean for 2.5% O_2 plus air.

When tomatoes were held in 5% and 2.5% O_2 for 27 days they attained color grade 3.8 and 3.7, respectively, and the cumulative

Table 8. Cumulative values of hourly rates (µl/kg-hr) of ethylene produced by "Walter" tomatoes at 20°C in 5% O_2, 2.5% O_2 and/or air for various periods.

Days after harvest*	Air	5% O_2	5% O_2 Transfer to air after 1 wk	5% O_2 Transfer to air after 2 wks	5% O_2 Transfer to air after 3 wks	2.5% O_2	2.5% O_2 Transfer to air after 1 wk	2.5% O_2 Transfer to air after 2 wks	2.5% O_2 Transfer to air after 3 wks
				µl/kg fr. wt.					
1	0.48	0.00	-	-	-	0.00	-	-	-
2	1.73	0.00	-	-	-	0.00	-	-	-
3	4.77	0.00	-	-	-	0.00	-	-	-
4	9.19	0.00	-	-	-	0.00	-	-	-
5	13.89	0.00	-	-	-	0.00	-	-	-
6	18.89	0.00	-	-	-	0.00	-	-	-
7	24.57	0.00	-	-	-	0.00	-	-	-
8	30.40	0.58	0.39	-	-	0.08	0.29	-	-
9	35.92	0.85	2.38	-	-	0.08	1.44	-	-
10	40.94	1.17	8.65	-	-	0.12	5.50	-	-
11	45.91	1.67	16.39	-	-	0.12	10.57	-	-
12	50.59	2.21	24.32	-	-	0.23	16.24	-	-
13	55.07	2.82	29.86	-	-	0.38	20.76	-	-
14	61.23	3.51	35.13	-	-	0.60	25.38	-	-
15	-	4.22	-	5.62	-	0.93	-	1.70	-
16	-	5.41	-	10.63	-	1.40	-	6.46	-
17	-	6.20	-	15.10	-	1.95	-	10.82	-
18	-	7.10	-	19.72	-	2.62	-	15.22	-
19	-	8.10	-	23.72	-	3.32	-	19.12	-
20	-	9.35	-	27.43	-	4.14	-	22.56	-
21	-	10.65	-	30.43	-	5.24	-	25.56	-
22	-	12.02	-	-	14.85	6.70	-	-	8.79
23	-	13.86	-	-	20.74	7.90	-	-	14.58
24	-	15.32	-	-	26.54	8.89	-	-	19.14
25	-	16.52	-	-	32.41	9.79	-	-	23.74
26	-	17.53	-	-	34.90	10.62	-	-	28.63
27	-	18.57	-	-	37.14	11.43	-	-	32.05
28	-	19.65	-	-	39.02	12.28	-	-	35.20

Note: Cumulative values are derived from Table 3 by adding the hourly rates (µl/kg-hr) of ethylene produced. Multiply by 24 to get cumulative daily production.

*Harvest day = June 27, 1973.

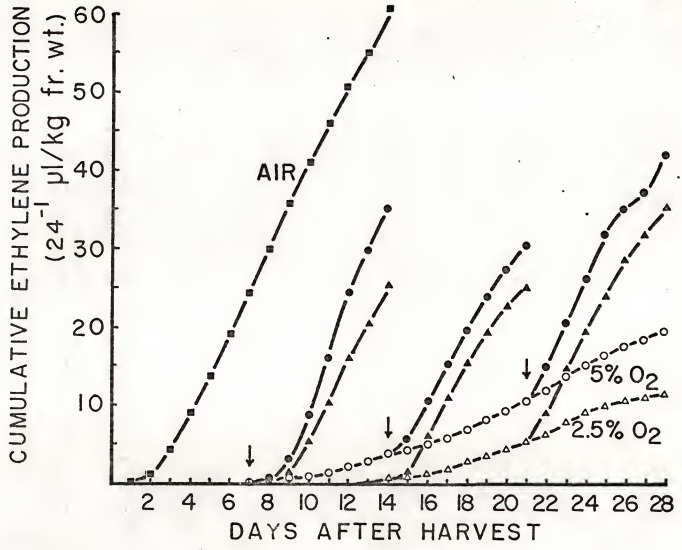


Figure 6. Cumulative values of hourly rates of ethylene by tomatoes at 20°C in 5% O_2, 2.5% O_2 and/or air. Multiply cumulative hourly rates by 24 to get cumulative daily production. Arrows indicate transfer of fruit from low oxygen treatment to air.

ethylene production reached 445.68 and 274.32 µl/kg, respectively. Thus, the fruit colored at the same rate in both oxygen concentrations, but much more ethylene was produced in 5% O_2 than in 2.5% oxygen.

Anatomical Development of Pigmentation

During normal tomato ripening in air at room temperature fruit coloring goes through the phases: mature-green, breaker, turning, pink, and red. This indicates that not all the tissues or cells of a fruit experience the same physiological age and ripening processes at the same time. However, there have been few reports to establish any definite sequential anatomical pattern of pigmentation of tomato tissues.

In low oxygen, color developed in radial stripes in the early stages (Fig. 7), with the pigmentation developing first (see Figs. 8 and 9) from the inner wall of the pericarp or columella and radial wall of the pericarp or interlocular septae, and then developing in the placental tissues and the outer wall of the pericarp. There was a clear distinction between outer and radial walls of the pericarp and outer and radial locular walls during early color development in low oxygen concentration (Fig. 8). The red stripes were comparatively more diffuse in 5% O_2 than in 2.5% O_2. Frequently, the dorsal vascular bundles were the last to lose greenness.

When mature-green tomatoes were transferred from low oxygen to air a faint orange color appeared all over the fruit which intensified with time (Fig. 8). No stripe pigmentation pattern showed up. This rapidly developing and uniform pigmentation which occurred after

Figure 7. Radial stripe color development of "Walter" tomatoes held 20 days in 2.5% O_2.

Figure 8.
The color of "Walter" tomato fruits held in 5% O_2, 2.5% O_2 or air for 25 days, or in 2.5% O_2 for 21 days plus 4 days in air.

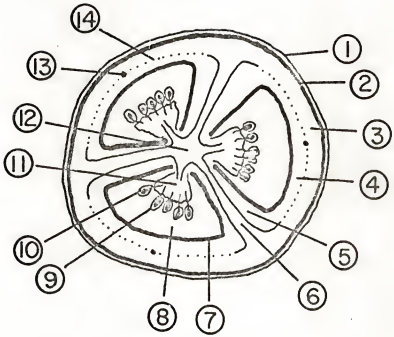


Figure 9. Schematic diagram of the anatomy of the cross section of a tomato fruit. (1) skin epidermis (a single cell layer); (2) skin collenchyma (3 or 4 cell layers); (3) outer wall of the pericarp; (4) locular wall of the pericarp; (5) radial wall of the pericarp; (6) interlocular septum; (7) locular endocarp; (8) locular cavity with jelly-like parenchyma around the seeds; (9) seed sac; (10) seed; (11) placental tissue; (12) inner wall of the pericarp, or columella; (13) dorsal or median vascular bundle; (14) vascular net.

transfer from low oxygen to air differed markedly from color development in air which was characterized by transitional color phases during which a fruit will have both green and pink areas at the same time.

Experiment II. Effect of Low Oxygen Concentration on Color Grade, on Firmness, and on Carotenoid and Flavonoid Synthesis

Effect of Continuous Low Oxygen Storage on Color Grade and Firmness

Color grade. Fruits reached color grade 5 by 14 days when held in air and by 35 days when held in 5% O_2 (Table 9, Fig. 10). When held in 2.5% O_2, the fruit only attained the grade of 4.2 in 49 days. Some color developed in fruits in 5% O_2 during the first 7 days whereas no color developed in fruits held in 2.5% O_2 for 21 days. The slow coloring in low oxygen was characterized by distinct radial stripes of pink-red color (Fig. 7) in the early storages (more distinct in 2.5% O_2 than in 5% O_2).

Firmness. Fruit held in 5% O_2 had softened to the same extent as fruit in air upon reaching color grade 5 (Table 10, Fig. 11). Fruit in 2.5% O_2 softened slowly during the 49 day period but did not attain full ripeness, as determined by color development and degree of softening.

Effect of Transfer of Tomatoes from Low Oxygen Storage to Air or Hypobaric Condition on Color Grade and Firmness

Color grade. When transferred to air, tomatoes resumed normal color development (Table 11, Figs. 12-1, 12-2). As tomatoes started ripening a faint yellowish orange color appeared all over the fruit as the green color faded away. The yellow-orange color intensified

Table 9. The color grade values of "Walter" tomatoes held at 20°C in continuous air, 5% O_2 or 2.5% O_2.

Treatment	Days in low oxygen							
	0	7	14	21	28	35	42	49
Air	1.0	4.2	5.0					
	0.0	0.8	0.0					
5% O_2	1.0	1.2	1.3	2.0	4.0	5.0	5.0	
	0.0	0.4	0.5	1.1	0.0	0.0	0.0	
2.5% O_2	1.0	1.0	1.0	1.0	2.3	3.2	3.2	4.2
	0.0	0.0	0.0	0.0	0.5	0.4	0.4	0.4

Note: The upper and lower values stand for means and standard deviations, respectively. Each value consists of 6 individual measurements with 1 being green and 5 being red.

Table 10. Firmness values of "Walter" tomatoes held at 20°C in continuous air, 5% O_2 or 2.5% O_2.

Treatment	Days in low oxygen							
	0	7	14	21	28	35	42	49
Air	1.15	2.98	4.58					
	0.26	1.19	0.58					
5% O_2	1.15	1.45	1.57	2.50	3.57	4.68	4.38	
	0.26	0.38	0.39	0.96	0.21	0.96	0.29	
2.5% O_2	1.15	1.18	1.53	1.67	2.45	3.52	3.43	3.87
	0.26	0.26	0.27	0.67	0.44	0.51	0.85	0.71

Note: The upper and lower values stand for means and standard deviations, respectively. Each value consists of 6 individual measurements. The lower the value the firmer the fruit on a 0 to 7 scale.

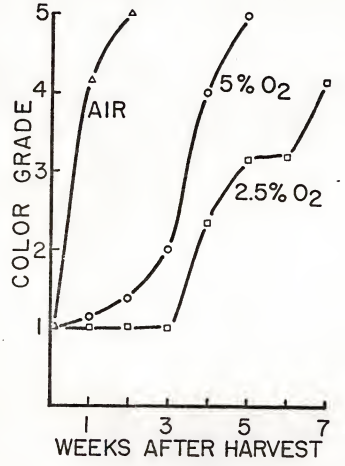


Figure 10. The effect of low oxygen atmosphere storage on the color development of tomatoes at 20°C. (Color grade: 1 = green, 5 = red.)

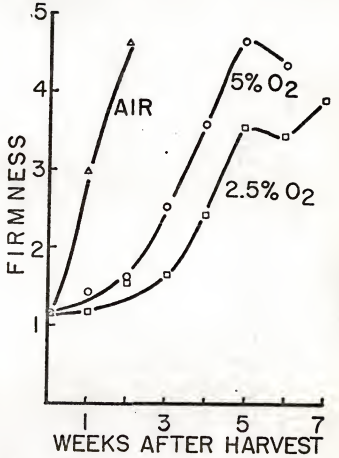


Figure 11. The effect of low oxygen atmosphere storage on the firmness of tomatoes at 20°C. The lower the value the firmer is the fruit on a 0 to 7 scale.

Table 11. The color grade values of tomatoes ripened in air or under hypobaric condition after being held in 5% and 2.5% O_2 for 7, 14, and 21 days.

Days in O_2	Days in air	5% O_2 Air	5% O_2 Hypobaric*	2.5% O_2 Air	2.5% O_2 Hypobaric
7	0	1.2 ± 0.4	1.2 ± 0.4	1.0 ± 0.0	1.0 ± 0.0
7	1	1.2 ± 0.4	1.0 ± 0.0	1.0 ± 0.0	1.2 ± 0.4
7	2	2.2 1.0	1.3 0.5	1.3 0.5	1.3 0.5
7	4	3.3 1.0	1.8 1.0	3.2 0.4	1.2 0.4
7	7	5.0 0.0	3.0 0.6	4.3 1.0	2.7 0.5
14	0	1.3 ± 0.5	1.3 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
14	1	2.0 0.9	2.5 ± 0.6	1.3 ± 0.5	1.0 ± 0.0
14	2	3.0 1.1	2.8 0.4	2.7 0.8	1.0 0.0
14	4	4.7 0.5	3.2 0.4	3.8 0.4	2.2 0.8
14	7	5.0 0.0	5.0 0.0	5.0 0.0	5.0 0.0
21	0	2.0 ± 1.1	2.0 ± 1.1	1.0 ± 0.0	1.0 ± 0.0
21	1	3.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
21	2	3.5 0.6	3.5 0.6	3.0 0.0	1.8 0.8
21	4	5.0 0.0	4.8 0.4	4.8 0.4	3.0 0.0
21	7	5.0 0.0	5.0 0.0	5.0 0.0	5.0 0.0

Note: Each value is the mean of 6 individual measurements with 1 being green and 5 being red. The standard deviations are listed in the second column.

*Hypobaric condition: 127 mm-Hg with air sweep of 250 ml/min at 20°C.

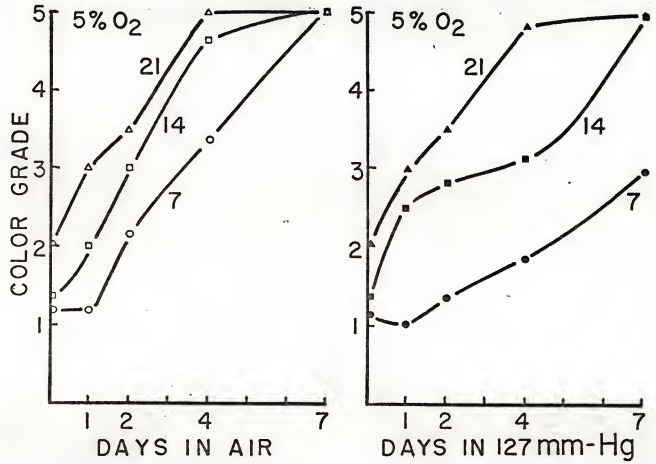


Figure 12-1. The color development of tomatoes after transfer to air or hypobaric condition (127 mm-Hg with air sweep) following 5% O_2 atmosphere storage for 7, 14, or 21 days at 20°C. (Color grade: 1 = green, 5 = red.)

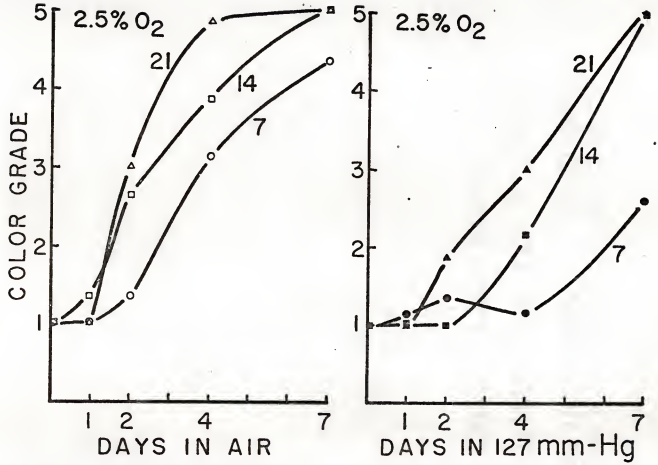


Figure 12-2. The color development of tomatoes after transfer to air or hypobaric condition (127 mm-Hg with air sweep) following 2.5% O_2 atmosphere storage for 7, 14, or 21 days at 20°C. (Color grade: 1 = green, 5 = red.)

until the tomato attained deep orange-red color (Fig. 8). Skin color also developed.

Tomatoes stored in 5% O_2 for 7 days had a 1-day lag period before coloring started and the rate of coloring was lower than that of tomatoes stored for 14 or 21 days (Fig. 12-1). However, they all attained color grade 5 in 7 days in air. Coloring was suppressed after transfer to hypobaric condition especially when the fruits were stored in 5% O_2 for 7 days before transfer (Fig. 12-1). The extent of suppression was less pronounced as the duration of low oxygen storage increased. Tomatoes stored in 5% O_2 for 21 days had already started coloring (color grade 2.0) at transfer and the hypobaric condition was not effective in suppressing the coloring.

Tomatoes stored in 2.5% O_2 were all of color grade 1.0 when transferred to air, however, the ones stored longer colored first (Fig. 12-2). Tomatoes from 21-day storage attained color grade 4.8 by 4 days. Those from 14-day storage attained color grade 5.0 by 7 days, while those from 7-day storage attained a color grade of only 4.3 by 7 days. The coloring response of these tomatoes to the transfer to air was not as rapid for the first few days as for those stored at 5% O_2. The suppression of tomato coloring by transfer to hypobaric condition was greater when tomatoes were stored in 2.5% O_2 than in 5% O_2 (Figs. 12-1, 2), and when tomatoes were held for a shorter period in low oxygen atmosphere.

Firmness. After being transferred to air or hypobaric condition for a week, fruits held in low oxygen for 7 days were firmer than fruits held 14 or 21 days (Table 12, Figs. 13-1, 2). The 21-day

Table 12. The firmness values of tomatoes ripened in air or under hypobaric condition after being held in 5% and 2.5% O_2 for 7, 14, and 21 days.

Days in O_2	Days in air	5% O_2 Air	5% O_2 Hypobaric*	2.5% O_2 Air	2.5% O_2 Hypobaric
7	0	1.45 ± 0.38	1.45 ± 0.38	1.18 ± 0.26	1.18 ± 0.26
7	1	1.35 ± 0.28	1.40 ± 0.23	1.38 ± 0.31	1.18 ± 0.21
7	2	1.47 ± 0.48	1.40 ± 0.24	1.33 ± 0.21	1.38 ± 0.28
7	4	2.50 ± 1.19	2.22 ± 0.79	2.20 ± 0.58	1.73 ± 0.28
7	7	4.00 ± 0.89	3.18 ± 0.96	3.25 ± 1.21	3.48 ± 1.50
14	0	1.57 ± 0.39	1.57 ± 0.39	1.53 ± 0.27	1.53 ± 0.27
14	1	2.00 ± 0.51	2.20 ± 0.41	1.40 ± 0.32	1.52 ± 0.21
14	2	2.18 ± 0.53	2.75 ± 0.55	1.92 ± 0.41	1.65 ± 0.21
14	4	3.68 ± 1.15	3.68 ± 0.72	2.75 ± 0.27	3.00 ± 0.87
14	7	6.03 ± 1.03	5.20 ± 0.84	4.65 ± 0.62	5.65 ± 0.51
21	0	2.50 ± 0.96	2.50 ± 0.96	1.83 ± 0.56	1.83 ± 0.56
21	1	2.88 ± 0.68	3.42 ± 0.49	2.05 ± 0.77	2.20 ± 0.30
21	2	3.70 ± 0.37	3.92 ± 0.95	2.43 ± 0.37	2.25 ± 0.19
21	4	4.07 ± 0.69	4.68 ± 0.96	3.17 ± 0.34	3.13 ± 0.68
21	7	4.65 ± 0.86	4.85 ± 0.50	3.82 ± 0.55	4.60 ± 0.95

Note: Each value is the mean of 6 individual measurements. The lower the value the firmer is the fruit on a 0 to 7 scale. The standard deviations are listed in the second column.

*Hypobaric condition: 127 mm-Hg with air sweep of 250 ml/min, at 20°C.

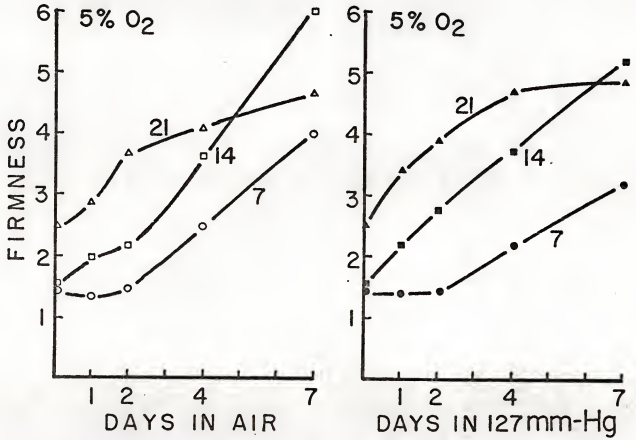


Figure 13-1. Firmness changes of tomatoes after transfer to air or hypobaric condition (127 mm-Hg with air sweep) following 5% O_2 atmosphere storage for 7, 14, or 21 days at 20°C. The lower the value the firmer the fruit on a 0 to 7 scale.

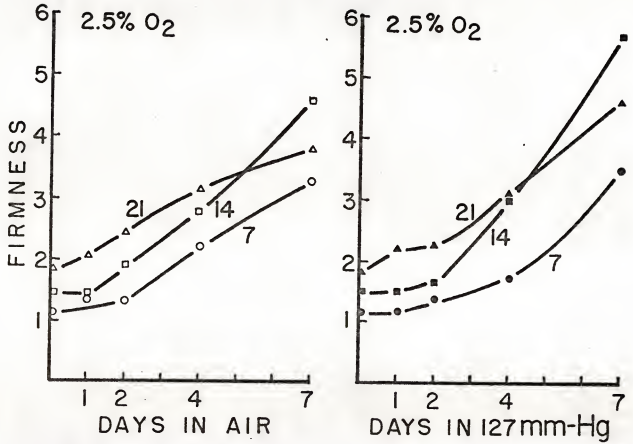


Figure 13-2. Firmness changes of tomatoes after transfer to air or hypobaric condition (127 mm-Hg with air sweep) following 2.5% O_2 atmosphere storage for 7, 14, or 21 days at 20°C. The lower the value the firmer the fruit on a 0 to 7 scale.

fruit were firmer than the 14-day fruit. Fruits transferred to air from 2.5% O_2 after 7, 14, or 21 days were firmer than fruits transferred to hypobaric condition. The reverse occurred for fruits held in 5% O_2 except for the 21-day fruits. Fruits transferred to air for one week from 2.5% O_2 were firmer than fruits from 5% O_2 even at comparable color grade values.

Carotenoid Contents in Air and Low Oxygen Atmosphere

Carotenes of "Walter" tomatoes. The carotenes and their concentrations found in ripe "Walter" fruit are shown in Table 13. The data are the means of six analyses of a single homogenate as a test of the precision of the procedure. The position and color of the carotenes on the developed column are shown in Table 14, and their spectral absorption curves are given in Appendix Figures 15 to 23.

One carotene was found in the extracts that appeared on the column in the position where either proneurosporene or prolycopene occur. The spectral absorption curves (Appendix Fig. 18) more closely fit the curves of proneurosporene than prolycopene as reported by Trombly and Porter (1953) and Qureshi *et al.* (1974a). In addition the color was yellow rather than brownish-orange of prolycopene (Porter and Zscheile, 1946). No further characterizaiton was made and the pigment is tentatively identified as proneurosporene and will be identified as such herein. Neither proneurosporene ncr prolycopene have been reported in red tomatoes but are constituents of tangerine types.

Carotene content of the tomatoes ripened in air and low oxygen atmosphere. Because of the lengthy analytical procedure, samples were

Table 13. The carotenoid content of the full ripe tomato fruit, cv. Walter. Six samples from the homogenate of an identical fruit were separately extracted and analyzed to determine the repeatability of the analyses.

Pigment	Content	Pigment	Content
Phytoene	15.29 ± 0.34	Neurosporene*	-
Phytofluene	3.66 0.17	Lycopene	25.90 ± 0.77
ζ-Carotene	1.47 0.08	γ-Carotene	0.92 0.11
Proneurosporene	0.52 0.10	β-Carotene	2.59 0.09

Note: Each value is the mean of 6 analyses of a single homogenate. The standard deviations are listed in the second column.

*Not determined.

Table 14. Position and color of carotene and colorless polyenes of tomato fruit cv. Walter on a Magnesia-Super Cel chromatographic column.

Position and Color	Substance
Red	all-*trans*-lycopene
Orange	unidentified
Clear space	
Yellow-orange	neurosporene
Clear space	
Red-orange	γ-carotene
Clear space	
Yellow	proneurosporene
Clear space	
Greenish yellow	ζ-carotene
Clear space	
Red-orange	β-carotene
Blue-green fluorescence	phytofluene
Colorless	phytoene

selected from the samples prepared from Experiment II for the comparison of carotene content.

Set I. Random samples representing color grade 1 through 5 were selected from lots ripened in air.

Set II. Tomatoes stored continuously in low oxygen atmosphere or in air.

(1) Air (0/14), Deep red: It represents "full-red" stage which is one more step beyond "red" stage of "Set I."

(2) 2.5% O_2 (42/0), Pink-red: Stored continuously in 2.5% O_2 for 42 days.

(3) 5% O_2 (28/0), Pink-red: Stored continuously in 5% O_2 for 28 days.

(4) 5% O_2 (42/0), Deep pink-red: Stored continuously in 5% O_2 for 42 days.

Set III. Tomatoes transferred to air after 14-day storage in 2.5% O_2.

(1) 2.5% O_2/air (14/1), Green: Held 1 day in air after 14 days in 2.5% O_2.

(2) 2.5% O_2/air (14/2), Pale orange-green: Held 2 days in air after 14 days in 2.5% O_2.

(3) 2.5% O_2/air (14/4), Yellow-orange: Held 4 days in air after 14 days in 2.5% O_2.

(4) 2.5% O_2/air (14/7), Deep orange-red: Held 7 days in air after 14 days in 2.5% O_2. Look like normal red ripe tomatoes, but has somewhat of an orange shade.

The carotene concentrations found in fruits in the three sets analyzed are presented in Tables 15-1, 15-2, and 15-3. The Duncan's multiple range indicies for these tables are given in Table 16. For easier direct comparison, the results from the low oxygen, low oxygen plus air and air treatments are placed together in Table 17. Several comparisons of these treatments are presented in bar graphs as Figures 14-1, 14-2, and 14-3.

Carotene changes during normal ripening in air. Only phytoene and β-carotene were detected in mature-green fruit (Table 15-1). At the breaker stage, a small quantity of lycopene was also detected. Phytofluene, ζ-carotene, and γ-carotene appeared in measurable amounts at the turning stage. Traces of proneurosporene and neurosporene were detected at the pink stage with larger amounts at the ripe stage. Although all pigments increased with ripening, the rate of increase differed considerably. Phytoene increased about 8 fold while β-carotene increased only 2.5 fold from the mature-green to the red stage.

Phytoene and lycopene were found in largest amounts in red fruit. Both increased markedly in the pink and red fruit. Phytofluene was the third largest in quantity in red fruits.

Somewhat similar results were obtained by Meredith and Purcell (1966) with "Homestead" tomatoes. One of the greatest differences was that they did not detect phytoene until the turning stage.

Carotene levels after long periods in low oxygen. Fruits held in 5% O_2 for 28 days or in 2.5% O_2 for 42 days had similar quantities of each carotene (Table 15-2). Fruit held in 5% O_2 for 42 days had much greater amounts of each carotene except β-carotene.

Table 15-1. The change in carotenoid content of "Walter" tomatoes during ripening in air at 20°C.

Stage of ripeness	Color grade	Carotenes * (µg/g fr. wt.) PE	PF	Z	PN	N	L	G	B
Mature-green	1	1.64	-	-	-	-	-	-	0.73
		0.43							0.32
Breaker	2	1.76	-	-	-	-	0.29	-	0.87
		1.48					0.11		0.31
Turning	3	1.12	0.21	0.10	-	-	1.58	0.18	1.07
		0.38	0.12	0.03			1.05	0.05	0.15
Pink	4	9.64	0.56	0.23	tr	tr	3.44	0.33	1.60
		8.27	0.28	0.21			1.18	0.05	0.25
Red	5	12.69	3.55	0.91	0.36	0.18	15.10	0.55	1.79
		3.14	1.74	0.39	0.05	0.15	5.87	0.19	0.79

Note: 1. The upper values are the means of the analyses of 4 individual fruits.
2. The lower values are the standard deviations.
3. The trace (tr) is less than 0.10 µg/g, and the dash (-) is not detectable.

*Carotenes: PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, B β-carotene.

Table 15-2. The carotenoid content of "Walter" tomatoes ripened in air or held in low oxygen atmosphere for different durations.

Oxygen level	Days in O_2	Days in air	Carotenes* (µg/g fr. wt.) PE	PF	Z	PN	N	L	G	B
Air	0	14	22.32	4.87	2.12	0.82	0.42	23.62	0.62	1.96
			1.44	0.13	0.24	0.18	0.22	4.29	0.09	0.64
2.5% O_2	42	0	7.91	0.75	0.18	0.15	0.13	16.88	0.56	3.30
			3.41	0.18	0.06	0.10	0.06	2.84	0.13	0.29
5% O_2	28	0	10.04	0.71	0.21	0.16	0.11	16.08	0.60	3.43
			9.08	0.26	0.08	0.04	0.04	3.58	0.19	1.66
5% O_2	42	0	13.42	3.02	1.46	0.48	0.24	31.22	0.73	3.72
			2.84	0.94	0.63	0.19	0.12	3.43	0.12	1.50

Note: 1. The upper values are the means of the analyses of 4 individual fruits.
2. The lower values are the standard deviations.

*Carotenes: PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, B β-carotene.

Table 15-3. The carotenoid content of "Walter" tomatoes ripened in air after 14-days' storage in 2.5% O_2 atmosphere.

Oxygen level	Days in O_2	Days in air	Carotenes* (µg/g fr. wt.) PE	PF	Z	PN	N	L	G	B
2.5% O_2/air	14	1	1.71	0.10	tr	-	-	-	-	0.61
			0.34	0.05						0.21
2.5% O_2/air	14	2	2.18	0.16	tr	-	tr	0.57	0.12	0.84
			0.40	0.06				0.47	0.07	0.14
2.5% O_2/air	14	4	9.13	0.87	0.44	0.21	0.18	6.34	0.39	1.93
			3.43	0.25	0.12	0.03	0.04	2.04	0.09	0.94
2.5% O_2/air	14	7	14.06	3.31	1.57	0.69	0.41	15.88	0.61	1.90
			0.22	0.47	0.38	0.17	0.37	3.59	0.14	0.40

Note: 1. The upper values are the means of analyses of 4 individual fruits.
2. The lower values are the standard deviations.
3. The trace (tr) is less than 0.10 µg/g, and the dash (-) is not detectable.

*Carotenes: PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, B β-carotene.

Table 16. Duncan's multiple range comparisons for the carotenoid means of Tables 13-1, 13-2, and 13-3.

Stage of ripeness or oxygen level	Days in O_2	Days in air	Carotenes* PE	PF	Z	PN	N	L	G	B
Table 13-1										
Mature grene			c	c	d	d	c	e	d	bc
Breaker			c	c	d	d	c	e	d	bc
Turning			c	c	d	d	c	e	cd	bc
Pink			b	c	d	d	c	de	bc	bc
Red	0	7+	b	b	c	bc	abc	c	ab	bc
Table 13-2										
Air	0	14	a	a	a	a	a	b	ab	b
2.5% O_2	42	0	b	c	d	d	abc	c	ab	a
5% O_2	28	0	b	c	d	d	bc	c	ab	a
5% O_2	42	0	b	b	b	b	abc	a	a	a
Table 13-3										
2.5% O_2/air	14	1	c	c	d	d	c	e	d	c
	14	2	c	c	d	d	c	e	cd	bc
	14	4	b	c	cd	cd	abc	d	bc	b
	14	7	b	b	b	a	ab	c	ab	b

Note: The same letters within a column indicate the means are not significantly different at the 5% probability level.

*Carotenes: PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, B β-carotene.

+Estimate of days to this red stage.

Table 17. Carotene content and color of tomato fruit (cv. Walter) held in 5% O_2, 2.5% O_2 and/or air for various periods.

Oxygen level	Days in O_2	Days in air	Carotenes* (µg/g fr. wt.) PE	PF	Z	PN	N	L	G	B	Fruit color
Air	0	7+	12.69 b	3.55 b	0.91 c	0.36 bc	0.18 ab	15.10 c	0.55 ab	1.79 b	Red
Air	0	14	22.32 a	4.87 a	2.21 a	0.82 a	0.42 a	23.62 b	0.62 ab	1.96 b	Deep red
2.5% O_2	42	0	7.91 b	0.75 c	0.18 d	0.15 d	0.13 ab	16.88 c	0.56 ab	3.30 a	Pink-red
5% O_2	28	0	10.04 b	0.71 c	0.21 d	0.16 d	0.11 b	16.08 c	0.60 ab	3.43 a	Pink-red
5% O_2	42	0	13.42 b	3.02 b	1.46 b	0.48 b	0.24 ab	31.22 a	0.73 a	3.72 a	Deep pink-red
2.5% O_2/air	14	4	9.13 b	0.87 c	0.44 cd	0.21 cd	0.18 ab	6.34 d	0.39 b	1.93 b	Yellow-orange
2.5% O_2/air	14	7	14.06 b	3.31 b	1.57 b	0.69 a	0.41 ab	15.88 c	0.61 ab	1.90 b	Deep orange-red

Note: 1. Each value is the mean of the analyses of 4 individual fruits.
2. The same letters within a column indicate the means are not significantly different at the 5% probability level.

*Carotenes: PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, B β-carotene.

+Estimate of days to this red stage.

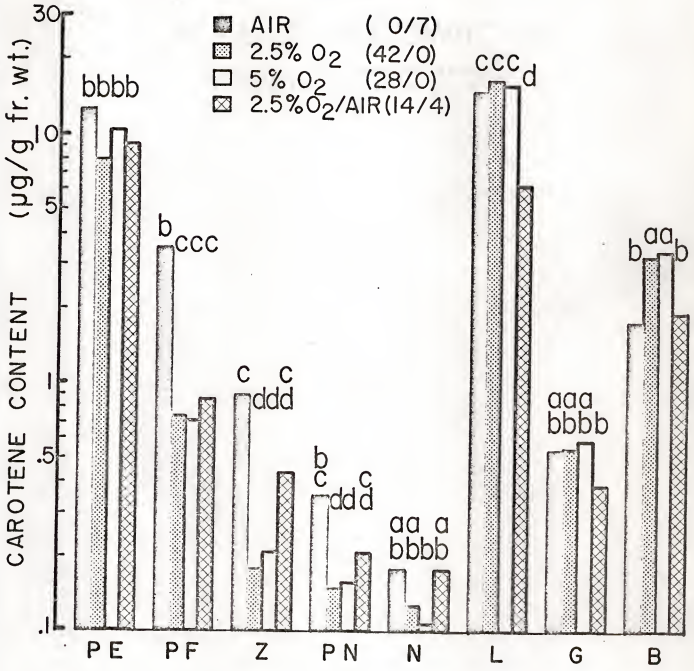


Figure 14-1. Carotene content of tomato fruit (cv. Walter) held in low oxygen and/or air. PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, and B β-carotene. The same letters within a carotene indicate the means are not significantly different at the 5% probability level. Comparisons are made across Figs. 14-1, 14-2, and 14-3.

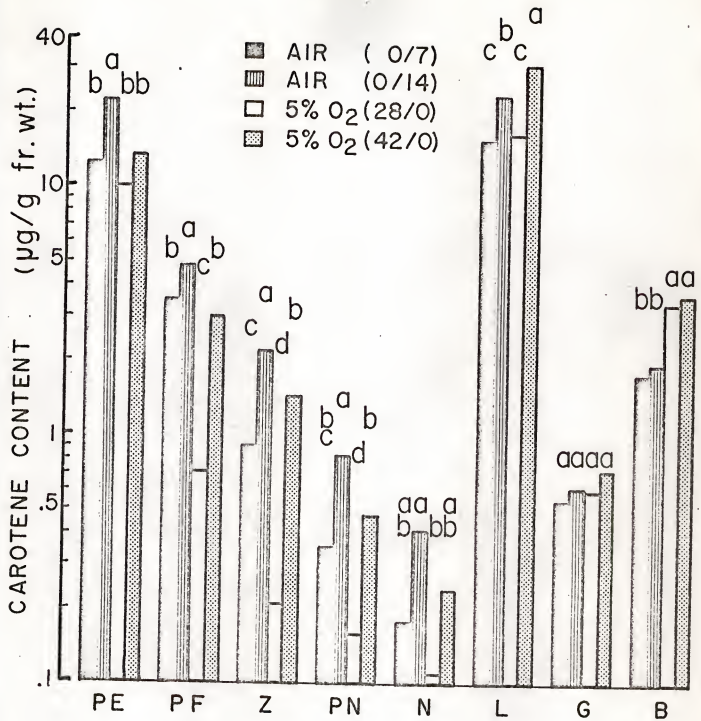


Figure 14-2. Carotene content of tomato fruit (cv. Walter) held in air or 5% O_2. PE phytoene, PF phytofluene, Z ζ-carotene, PN pro-neurosporene, N neurosporene, L lycopene, G γ-carotene, and B β-carotene. The same letters within a carotene indicate the means are not significantly different at the 5% probability level. Comparisons are made across Figs. 14-1, 14-2, and 14-3.

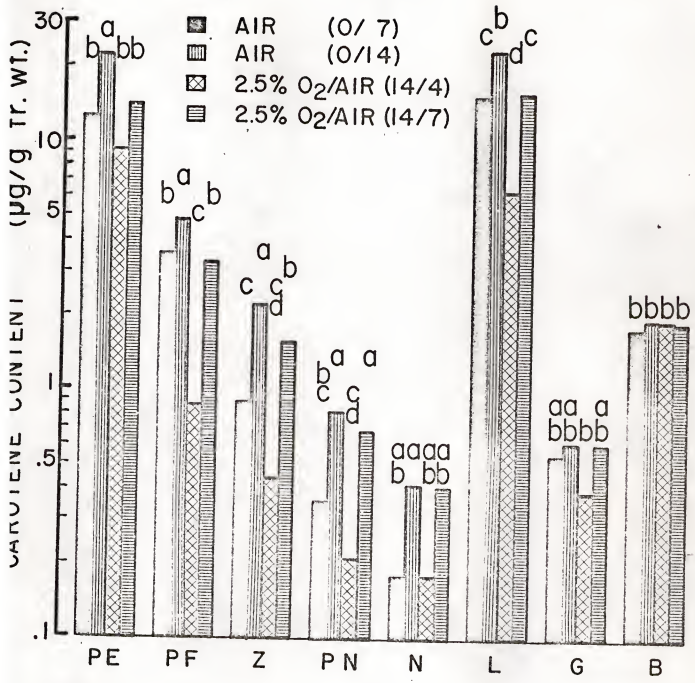


Figure 14-3. Carotene content of tomato fruit (cv. Walter) held in low oxygen and/or air. PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, and B β-carotene. The same letters within a carotene indicate the means are not significantly different at the 5% probability level. Comparisons are made across Figs. 14-1, 14-2, and 14-3.

Carotene levels in fruit transferred to air after 14 days in 2.5% O_2. The carotenoid development during ripening was similar to that of fruit ripened in air (Table 15-1) except that phytofluene, ζ-carotene, proneurosporene, neurosporene, and γ-carotene appeared sooner during ripening (Table 15-3).

Comparison of carotene levels of fruit in low oxygen with fruit ripened in air. The color of the fruit held in 2.5% O_2 for 42 days or 5% O_2 for 28 days were pink-red. Fruit held in 5% O_2 for 42 days were deep pink-red. After ripening 7 days in air following 14 days at 2.5% O_2, the fruit color was deep orange-red.

The fruits ripened in air for 14 days, which were overripe, had higher levels of phytoene, phytofluene, ζ-carotene, proneurosporene, and lycopene than red fruits ripened in air (Table 17, Fig. 14-2). Likewise, tomatoes held in 5% O_2 for 42 days accumulated higher amounts of phytofluene, ζ-carotene, proneurosporene, and lycopene than those held in 5% O_2 for 28 days (Table 17, Fig. 14-2). Also, tomatoes ripened in air for 7 days following 14 days in 2.5% O_2 had higher amounts of phytofluene, ζ-carotene, proneurosporene, and lycopene than those ripened in air for 4 days following 14 days in 2.5% O_2 (Table 17, Fig. 14-3).

Phytoene, neurosporene, lycopene and γ-carotene contents of the fruits held in 2.5% O_2 for 42 days or in 5% O_2 for 28 days were not significantly different from those of red fruits ripened in air (Table 17, Fig. 14-1). However, the former had only 20% as much phytofluene or ζ-carotene, and less than 50% as much proneurosporene as the latter,

whereas there was nearly 50% more β-carotene in the low oxygen fruits than in the red.

Tomatoes held in 5% O_2 for 42 days had 32% more lycopene and 90% more β-carotene than tomatoes ripened in air for 14 days (Table 17, Fig. 14-2). However, the former had only 60% as much phytoene, 62% as much phytofluene, 66% as much ζ-carotene, and 59% as much proneurosporene as the latter.

Thus, when tomatoes ripened slowly in low oxygen atmosphere β-carotene accumulated more than in air, and there was significantly less phytofluene, ζ-carotene, and proneurosporene than in air even though the lycopene content was the same or even higher than in air.

Comparison of carotene levels of fruit transferred to air after 14 days in 2.5% O_2 with fruit ripened in air. Tomatoes ripened in air for 4 days following 14 days in 2.5% O_2 had only 42% as much lycopene and 25% as much phytofluene as red fruit (Table 17, Figs. 14-1 and 14-3). There was no significant difference in phytoene, ζ-carotene, proneurosporene, neurosporene, γ-carotene, or β-carotene although these, except neurosporene and β-carotene, were lower in the former.

Tomatoes ripened in air for 7 days following 14 days in 2.5% O_2 had 73% more ζ-carotene and 92% more proneurosporene than red (air) fruit (Table 17, Fig. 14-3). There was no significant difference in other carotenes including lycopene and β-carotene.

Thus, when tomatoes ripened upon transfer to air as mature-green fruits after 14 days of low oxygen storage, β-carotene level was not affected, but ζ-carotene and proneurosporene accumulated more than in the control (air) even with the same level of lycopene.

Lycopene to ζ-carotene or lycopene to ζ-carotene, proneurosporene plus neurosporene ratio as an index for the modification of the tomato color. The total carotenoid to β-carotene ratio has been used as a means of judging red color with the redder fruits having the higher ratio. However, a comparison of the lycopene/β-carotene ratio (lycopene being the largest contributor to total carotenoids) shows (Table 18) that the pink-red fruit had a much lower ratio than the red fruit which is the reverse of the expected. However, if the comparisons are made of the ratios of lycopene to ζ-carotene or lycopene to ζ-carotene plus proneurosporene plus neurosporene the pink-red fruits are found to have much higher ratios than the red fruits.

The low levels of ζ-carotene which is greenish yellow and proneurosporene which is yellow (see Table 14) may be why tomatoes ripened slowly in low oxygen were pink-red rather than red. This modification of tomato fruit color is reflected in the ratio of the red pigment lycopene to the yellow pigments ζ-carotene, proneurosporene, and neurosporene (Table 18). The ratio L/(Z+PN+N) was more than 3 times greater in the fruit held in 2.5% O_2 for 42 days or in 5% O_2 for 28 days than in red fruit ripened in air. The ratio in the fruit held in 5% O_2 for 42 days was 1.4 times greater than in the red fruit, and 2.1 times greater than in the fruit ripened in air for 14 days.

The higher levels of ζ-carotene and proneurosporene may be why tomatoes ripened in air for 7 days after 14 days at 2.5% O_2 were deep orange-red rather than red. The ratio L/(Z+PN+N) of the above fruits was only 57% as much as that of the red fruit (Table 18). Thus the modification of the color quality of tomato fruits in low oxygen

Table 18. The ratio of various carotenes of tomato fruit (cv. Walter) held in 5% O_2 2.5% O_2 and/or air for various periods.

Oxygen level	Days in O_2	Days in air	Lycopene	Ratio* PE/PF	PF/Z	Z/N	Z/PN	B/G	L/B	L/PE	L/Z	$\frac{L}{Z+PN+N}$
			(μg/g)									
Air	0	7+	15.10	3.57	3.90	5.06	2.53	3.25	8.44	1.19	16.59	10.41
Air	0	14	23.62	5.58	2.20	5.26	2.70	3.16	12.05	1.06	10.69	6.85
2.5% O_2	42	0	16.88	10.55	4.17	1.38	1.20	5.89	5.12	2.13	93.77	36.70
5% O_2	28	0	16.08	14.14	3.38	1.91	1.31	5.72	4.69	1.60	76.57	33.50
5% O_2	42	0	31.22	4.44	2.07	6.08	3.04	5.10	8.39	2.33	21.38	14.32
2.5% O_2/air	14	4	6.34	10.49	1.98	2.44	2.10	4.95	3.28	0.69	14.41	7.64
2.5% O_2/air	14	7	15.88	4.25	2.11	3.83	2.28	3.11	8.36	1.13	10.11	5.95

Note: This table is derived from Tables 15-1, 15-2, and 15-3.

*Ratio: PE phytoene, PF phytofluene, Z ζ-carotene, PN proneurosporene, N neurosporene, L lycopene, G γ-carotene, B β-carotene.

+Estimate of days to this red stage.

storage may be a reflection of the change in the carotenoid composition.

The ratios between carotenes. A stepwise decrease of the carotene levels was observed along the dehydrogenation steps, phytoene (PE) - phytofluene (PF) - ζ-carotene (Z) - neurosporene (N); and the side branch, ζ-carotene (Z) - proneurosporene (PN) (Tables 15-1, 15-2, and 15-3). The substrate to product ratios (PE/PF, PF/Z, Z/N, and Z/PN) varied depending upon the ripening stage and the oxygen level of the storage atmosphere, but approached values ranging from 2 to 6 (Table 18).

There was a stepwise increase from γ-carotene to β-carotene (Tables 15-1, 15-2, and 15-3). The ratio B/G decreased with time (Tables 15-1, 15-2, and 15-3) to approach a value about 3 when tomatoes ripened in air, but about 5 when tomatoes ripened in low oxygen only (Table 18).

Lycopene accumulated at a high level and was not related to the stepwise decrease or increase observed with the other carotenes (Tables 15-1, 15-2, and 15-3). However, the level of lycopene matched closely that of phytoene especially as tomatoes approached ripeness (Table 18). The comparison of lycopene to phytoene (L/PE) ratio between tomatoes with the same lycopene content showed that the ratio was higher in low oxygen atmosphere than in air: the ratio L/PE of the red fruit (air) was 1.19, and that of the fruit ripened in air for 7 days following 14 days in 2.5% O_2 was 1.13, whereas that of the fruit held in 2.5% O_2 for 42 days was 2.13 and that of the fruit held in 5% O_2 for 28 days was 1.60.

Effect of Low Oxygen on Tomato Skin Color

During normal ripening a yellow flavonoid pigment develops in the tomato skin (Table 19). However, in low oxygen storage, even though carotenoids were synthesized in the flesh, the skin pigment did not develop until after 30 to 40 days when some color appeared around the stem and stylar ends of the fruit.

Normal skin color developed when the fruit were transferred to air after short periods of low oxygen but not after long periods of low oxygen.

Table 19. The skin color score of tomatoes ripened in low oxygen and/or air for different durations.

Stage of ripeness or oxygen level	Days in O_2	Days in air	Skin color score[a,b]
Set I			
Mature green			1.0 ± 0.0
Breaker			1.0 ± 0.0
Turning			2.0 0.0
Pink			3.0 0.0
Red			4.0 0.0
Set II			
Air	0	14	5.0 ± 0.0
2.5% O_2	42	0	1.0 ± 0.0
5% O_2	28	0	1.0 0.0
5% O_2	42	0	1.5 0.5
Set III			
2.5% O_2/air	14	1	1.0 ± 0.0
2.5% O_2/air	14	2	1.0 ± 0.0
2.5% O_2/air	14	4	2.0 0.0
2.5% O_2/air	14	7	3.3 0.4

[a]1 = colorless skin, 5 = deep yellow skin.
[b]Means and standard deviations of 4 individual fruits.

DISCUSSION

Experiment I. Effect of Low Oxygen Concentration on Color Grade, and on CO_2 and Ethylene Production

Respiration, Ethylene Production and Coloring in Low Oxygen and Air

Tomato fruit colored slowly in low oxygen even though the typical climacteric with sharp increases in carbon dioxide and ethylene production did not occur. Actually, the respiration decreased to about half the beginning rate after a few days and was only about 65% of that rate after 24 days. Ethylene production, which was delayed several days, never reached a rate more than about 30% of that of fruits held in air. These results with tomatoes are similar to those obtained with bananas (Hesselman and Freebairn, 1969; Quazi and Freebairn, 1970), pears (Wang and Hansen, 1970), and potato tuber (Reid and Pratt, 1972) and stress the need for reconsidering the role of the respiratory climacteric in ripening.

Upon transfer to air from low oxygen storage, the fruit colored rapidly and exhibited a climacteric rise in CO_2 and ethylene production. A considerable increase in the CO_2 production rate occurred in the first day after transfer for each of the periods 7, 14, or 21 days. The ethylene production rate of fruit transferred to air after 7 days in low oxygen corresponded to fruit held in air being low for the first day. Fruit held in low oxygen for 7 and 14 days had much higher rates of ethylene production after one day in air than fruit held in air.

Little color development occurred the first day after transfer to air following 7 days in low oxygen which is similar to the ethylene results. However, there was no color development or ethylene production up to this time. In the case of the later transfers (14 and 21 days) color had begun to develop and ethylene was being produced before transfer to air.

The low oxygen storage seemed to have no detrimental effect on the ripening process. The rapid ripening response following transfer to air after the longer periods at low oxygen indicates that even in low oxygen atmosphere the biochemical facilities for tomato ripening were slowly built up so that they functioned fully after transfer to air. This is in line with the suggestion of Hulme *et al.* (1971) that a part of the ripening system producing the "enzymes of ripening" is being slowly built up during the low oxygen storage.

Quantitative Relationship of CO_2 and Ethylene to Ripening

The cumulative amounts as well as the rates of CO_2 and ethylene production were considered in this study since color development itself is an expression of quantity rather than of rate. The overall pattern of cumulative production of CO_2 and ethylene closely matched that of color grade (Figs. 2, 5, and 6).

The cumulative amounts of CO_2 and ethylene production tended to be related to the color grade attained but there were some inconsistencies particularly with ethylene. The major inconsistency with CO_2 production was that fruit held continuously in low oxygen produced as much CO_2 as fruit in the other treatments but were over one grade lower in color.

There were two inconsistencies with ethylene production and color grade. The major one was that fruit ripened in air produced 70 to 100% more ethylene than low oxygen plus air treatments to attain color grade 5. The other was the large difference in ethylene production of fruits held in 2.5% or 5% O_2 but which attained similar color grades.

It may be that the color grade method of measuring color is too subjective to make good quantitative comparisons. The use of multiple fruit samples with variability in numbers attaining various color grades would have considerable effect on the results also. The carbon dioxide and ethylene produced are averages of the rates of the individual fruit at any one time.

Whether a true relationship exists between ripeness and accumulated production of CO_2 and ethylene can only be determined by more experiments using single fruits and objective color measurements.

Development of Pigmentation

In low oxygen atmospheres, color developed in radial stripes at the beginning. This coloration pattern is not uncommon since it occasionally happens under natural conditions. It may reflect a transitional step in tomato ripening which was revealed because of the slow rate of ripening in low oxygen atmosphere. It may also indicate a difference between tissues in accessibility to oxygen or a difference between tissues in the sensitivity to ripening stimuli.

When mature-green tomatoes were transferred from low oxygen to air, faint orange color started to appear all over the fruit, and

intensified afterwards. No stripe pattern of pigmentation showed up. This may indicate that some part of the ripening process has developed during the period of low oxygen storage, but further development was held up by the oxygen limitation.

Experiment II. Effect of Low Oxygen Concentration on Color Grade, on Firmness, and on Carotenoid and Flavonoid Synthesis

Coloring and Softening of Tomatoes in Low Oxygen, Air, and Hypobaric Storage

In low oxygen storage, tomato coloring and softening were both inhibited initially, but progressed slowly thereafter. The yellow skin flavonoid did not develop in low oxygen atmosphere until about 30 to 40 days when yellow color developed in limited areas of floral and stem ends. The flesh carotenoids were slowly synthesized and the fruit became pink-red. After 42 days in 5% O_2, tomatoes became a deep red-pink, whereas in 2.5% O_2 they became only light pink. This modification of tomato coloration is in agreement with the observation made by Parsons *et al.* (1970). Salunkhe and Wu (1973) reported that there was no significant difference in lycopene and β-carotene content between the finally ripened fruits in air and in low oxygen atmosphere, and they concluded that this indicated that low oxygen atmosphere slowed ripening but did not affect the final color of the fruit. However, they did not analyze the other intermediates of the carotenoid biosynthetic pathyway which also contribute to the final color. Tomatoes developed a deep orange-red color when ripened in air after low oxygen storage.

When tomatoes were transferred at weekly intervals from low oxygen storage to air, they resumed normal color development and softening, after a one day lag period. There was no lag period when the fruit was held for long periods (about 3 weeks) in low oxygen during which time the fruit had produced a small but significant amount of ethylene for inducing ripening. The hypobaric condition was not effective in inhibiting ripening in this case, although it inhibited tomato coloring quite effectively when the fruit had been held in low oxygen for only one week, without ethylene production, before putting into the hypobaric system. This phenomenon is quite similar to the gradual reduction of the inhibitory effect of cycloheximide on pear ripening with application time (Frenkel *et al.*, 1968). Hulme *et al.* (1971) studied some biochemical changes of apple in low oxygen atmosphere. When the apple disks were transferred from 3% O_2 after 31 days to air, there was a rapid adjustment to the normal (in air) situation with increased respiration, protein synthesis, and the malate decarboxylating system. They suggested from the fact that there was no large increase in uridine incorporation as a preliminary to increased protein synthesis that transcription was building up to a point where translation was limited only by oxygen so that, on transfer to air, there was only a short lag before protein (enzyme) synthesis became stimulated.

Modification of Carotene Biosynthesis in Low Oxygen and Air

The occurrence of proneurosporene in "Walter" tomato was rather unexpected, since this pigment has been reported only in tangerine

genotype. The lack of prolycopene and the low levels of prolycopene and ζ-carotene compared to those of tangerine genotype (Mackinney and Jenkins, 1952; Tomes, 1963), however, suggest this variety be classified into the red genotype.

The modification of tomato fruit color in low oxygen atmosphere was shown in this study to be a reflection of the compositional changes in the carotenoid spectrum. The modification was not due to an inhibition at a specific step in the biosynthetic pathway, since the intermediates as well as lycopene and cyclic carotenes also kept building up in low oxygen atmosphere as the ripening progressed slowly. The modification was rather a matter of time and extent of production of the three seemingly different sets of carotenes, *i.e.* the intermediates (phytofluene through neurosporene), lycopene, and cyclic carotenes.

That β-carotene accumulates to higher level in lower than atmospheric partial pressure of oxygen is in agreement with the observation of Kushwaha *et al.* (1969) who showed that conversion of tritiated lycopene to cyclic carotenes by spinach plastids was greater in nitrogen than in air.

The idea that low oxygen partial pressure results in end product accumulation rather than intermediates is supported by the study of Jensen *et al.* (1958). They showed that the incubation of *Rhodospirillum rubrum* with diphenylamine (DPA) inhibited the net synthesis of the normal carotenoids (lycopene, P481, spirilloxanthin) and the more saturated carotenes (phytoene, phytofluene, ζ-carotene, and neurosporene) started to accumulate. If the DPA was removed and the cells

were resuspended in buffer and incubated anaerobically in the light, an endogenous synthesis of normal carotenoids took place at the expense of all the accumulated precursors (phytoene, ζ-carotene, and neurosporene).

The pattern of the compositional changes of the tomato carotenoids and the quantitative relationship between the components indicate that the intermediates (phytofluene, ζ-carotene, neurosporene, and proneurosporene as a side branch from ζ-carotene), lycopene, and cyclic carotenes (γ-carotene and β-carotene) might be functionally separate from each other. The intermediates exhibited a sequential decrease in concentration, and this relationship is in agreement with the results of Jensen *et al.* (1958), Anderson and Porter (1962), and Eslava and Cerdá-Olmedo (1974). Lycopene was the major pigment and its accumulation did not follow the sequential decrease of the intermediates nor was related to β-carotene. On the contrary, lycopene level was closely related to phytoene level. This suggests that there might exist an equilibrium between phytoene and lycopene.

It is interesting, in this relation, to note that there exist three ultrastructurally different entities in the chromoplasts of the tomatoes which might represent functionally different sets of the carotenogenic enzyme system. Harris and Spurr (1969a, b) reported that β-carotene of high-beta mutant is formed largely in the globules in the stroma, whereas in normal red tomatoes lycopene crystalloids develop in thylakoids. Of particular interest is the thylakoid plexus which occurs in chromoplasts of tangerine tomatoes (Rosso, 1967).

Such a thylakoid plexus occurs also in high-beta strain and normal red tomatoes (Harris and Spurr, 1969a, b). But such structures usually develop after the grana-inter-grana thylakoid system has undergone considerable change and the carotenoid pigments have increased during chromoplast development (Harris and Spurr, 1969a, b). It will be interesting to test the possibility that the thylakoid plexus might reflect the formation of the series of the intermediates and/or the side branch from ζ-carotene.

The mechanism of the modification of the carotenoid composition in low oxygen atmosphere remains to be answered. It could be the nitrogen which might possibly be protecting sulfhydryl group of the enzyme from oxidation as in the soluble extracts of spinach and tomato plastids (Kushwaha *et al.*, 1969), or it could be the reduced reaction rate with the lack of respiratory climacteric and the auto-catalytic ethylene production that is responsible for the modification.

Modification of Tomato Skin Flavonoid Biosynthesis in Low Oxygen Concentration

The synthesis of yellow skin color (quercitrin according to Wu and Burrell, 1958) of "Walter" tomato was inhibited in low oxygen storage. In this experiment light condition was not originally controlled. The fluorescent light of the ripening room was turned on during working hours (more than 2 hours a day) and the incandescent light was closely applied during every day's color scoring. Piringer and Heinze (1954) reported that the threshold value for the light requirement to produce the yellow cuticle pigment to be equivalent to

that supplied by an incandescent-filament lamp somewhere between 0.0005 and 0.005 fc for one hour, or 0.03 or 0.3 fc for one minute per day during the ripening period. They also used white fluorescent lamp with a red cellophane filter as a red light source. Thus, the lighting condition in this experiment should not be a limiting factor. It was observed that the fruits in air developed intense yellow skin color under the same lighting condition while the fruits in low oxygen storage remained colorless on the skin. The possible mechanism of the inhibition of skin flavonoid biosynthesis in low oxygen storage awaits further investigation. It is noteworthy that one molecule of quercitrin itself contains 11 atoms of oxygen, whereas hydrocarbon carotenoids contain no oxygen atom. It is also important to note that it is the molecular oxygen that is incorporated into the hydroxyl group of *p*-coumaric acid (Smith, 1972) and caffeic acid (Fritz *et al.*, 1974) which is an intermediate and a side branch of the pathway of flavonoid biosynthesis.

SUMMARY AND CONCLUSION

Ripening responses of tomatoes (cv. Walter) in low oxygen atmosphere (5% O_2 and 2.5% O_2 in N_2) as a continuous flow system were studied in terms of color grade, firmness, CO_2 and ethylene production, and carotenoid composition, and skin flavonoid synthesis. Ripening room was maintained at 20°C (68°F) and 90% relative humidity.

In 5% and 2.5% O_2 rates of CO_2 and ethylene evolution remained very low without any climacteric peak, whereas coloring, after 9 to 12 days' delay of onset, progressed slowly. When tomatoes were transferred to air after storage in low oxygen for 1, 2, or 3 weeks, rates of CO_2 and ethylene evolution increased rapidly to reach the normal level, and the color developed fully.

Although tomatoes in low oxygen lacked a respiration climacteric and an autocatalytic ethylene production, the total amounts of CO_2 and ethylene produced during the time it took for the fruit to reach a certain color grade strongly suggests that there are absolute quantitative requirements for ethylene and respiration for tomato ripening. Ethylene is not only necessary for triggering the onset of ripening, but might be also required continuously for subsequent ripening.

The pink-red color which slowly developed in 5% and 2.5% O_2 was attributed to the inhibition of biosynthesis of skin flavonol and changes in the composition of hydrocarbon carotenes. In low oxygen, lycopene and β-carotene accumulated with diminished production of

intermediates, *i.e.*, phytofluene, ζ-carotene, and proneurosporene. When mature-green fruits were transferred to air after low oxygen storage, tomatoes ripened rapidly concurrent with increased production of ethylene and respiration. The fruit color was deep orange-red rather than red as in normal, and this was attributed to the synthesis of the skin flavonol and changes of the composition of pericarp carotenes. This color change was associated with increased proportion of phytofluene, ζ-carotene, and proneurosporene.

APPENDIX

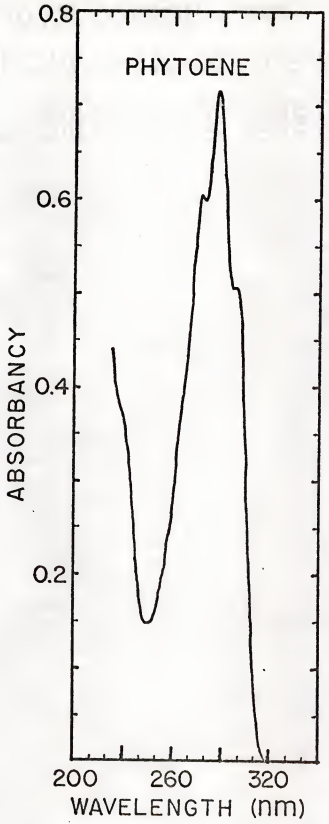


Figure 15. Spectral absorption curve of phytoene in hexane (λ_{max}: 296s, 286, 276; λ_{min}: 278 nm).

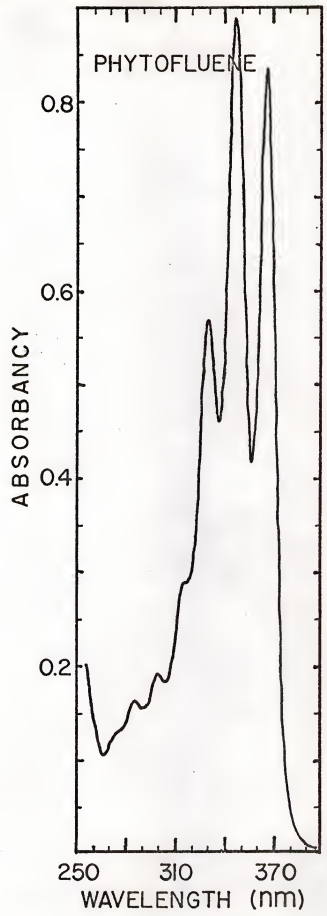


Figure 16. Spectral absorption curve of phytofluene in hexane (λ_{max}: 367, 347, 331; λ_{min}: 358).

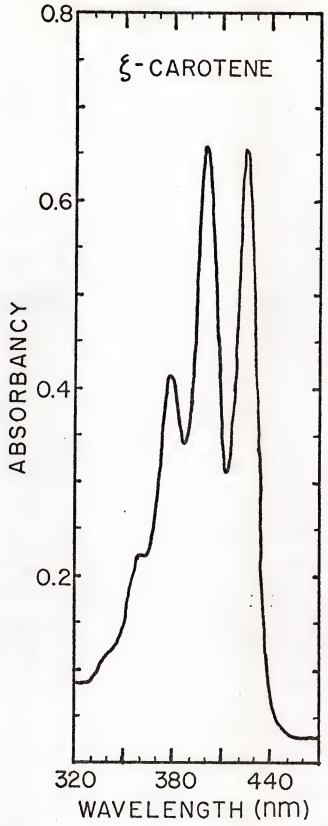


Figure 17. Spectral absorption curve of ζ-carotene in hexane (λ_{max}: 424, 399, 377; λ_{min}: 413, 387 nm).

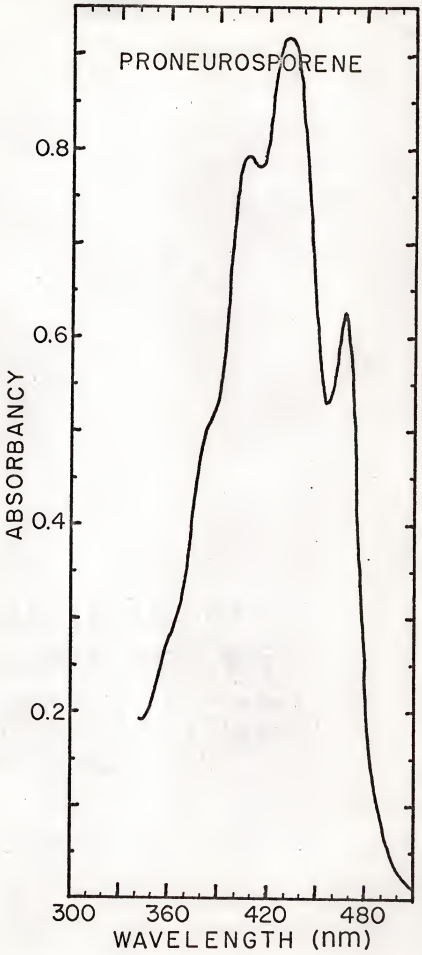


Figure 18.
Spectral absorption curve of proneurosporene in hexane (λ_{max}: 463, 428, 402; λ_{min}: 455, 415 nm).

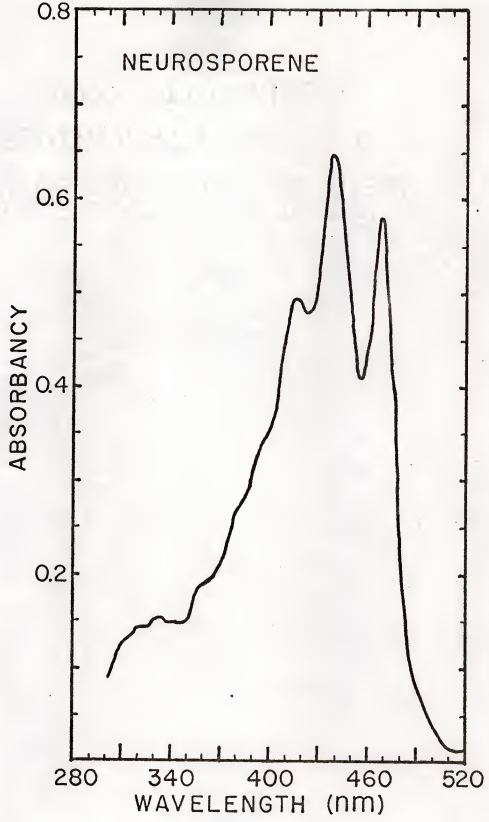


Figure 19. Spectral absorption curve of neurosporene in hexane (λ_{max}: 467, 438, 415; λ_{min}: 456, 423 nm).

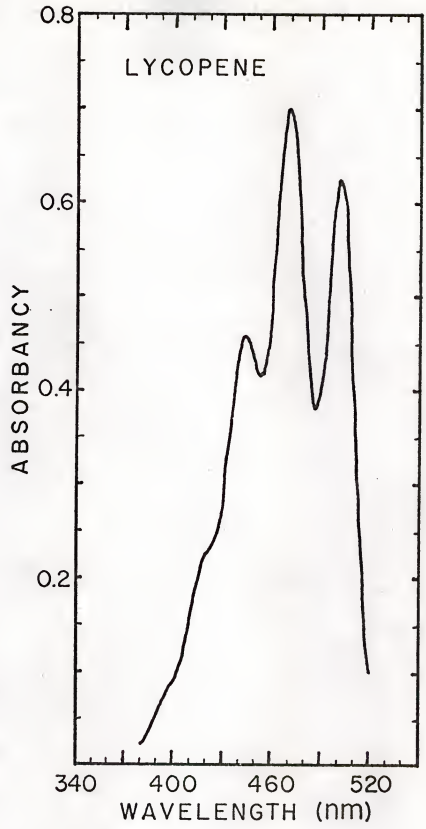


Figure 20. Spectral absorption curve of lycopene in hexane (λ_{max}: 500, 469, 442; λ_{min}: 485, 452 nm).

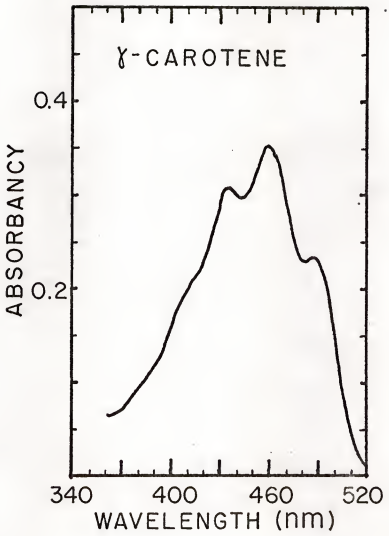


Figure 21. Spectral absorption curve of γ-carotene in hexane (λ_{max}: 480, 460, 435; λ_{min}: 482, 444 nm).

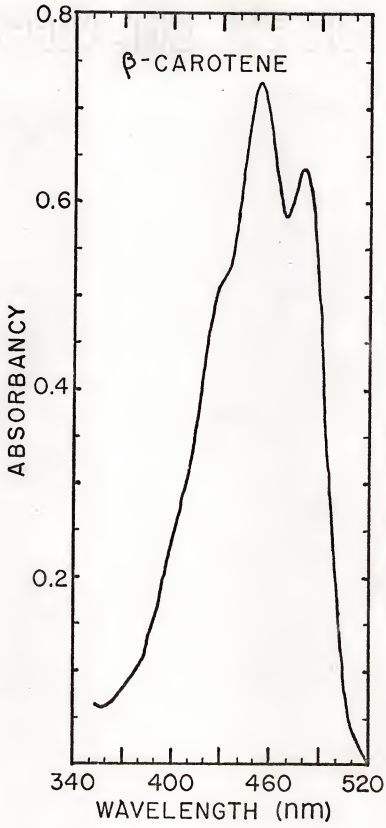


Figure 22. Spectral absorption curve of β-carotene in hexane (λ_{max}: 477, 449, 426s; λ_{min}: 465 nm).

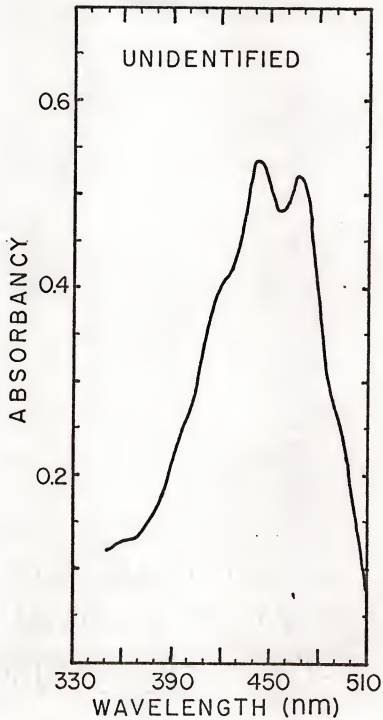


Figure 23. Spectral absorption curve of an unidentified carotene in hexane (λ_{max}: 468, 445, 425s; λ_{min}: 458 nm).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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